

Don't rush them to predict earthquakes

MORE people died in Turkey last Saturday as a result of an earthquake than have ever died in the United States from earthquakes. And yet earthquakes in the United States attract an enormous global attention. One reason is simply human curiosity that a technological leader can be laid low by acts of God; for much the same reason are medical bulletins of heads of state read with fascination. A more subtle reason is the extraordinary way in which the lessons of every new major earthquake have been very rapidly learnt, although no doubt one could argue that the biggest lesson of all—don't live in regions prone to earthquakes—has yet to be assimilated.

The United States represents, in very crude terms, much less than a thousandth of the world's earthquake fatality potential—first, because a relatively small proportion of the world's active fault system is located in the United States; second, and more important, because awareness of the earthquake hazard is higher and thus elementary defensive measures are more widely practised, particularly in construction. Few people die from the shaking itself; it is the falling of buildings, the collapse of dams, the starting of fires and so on which claims life.

There is a worldwide interest in the earthquake prediction programme in the United States (completely over-shadowing the arguably more interesting and important programmes in the Soviet Union and China). This interest is bound to grow with the publication of *Earthquake Prediction and Public Policy*, an extensive document from the Panel on the Public Policy Implications of Earthquake Prediction under the chairmanship of Professor Ralph Turner, a sociologist from the University of California, Los Angeles. The layers of bureaucracy in Washington are so thick that initials must suffice to describe the report's parentage and destination: it was prepared by the PPPIEP of the ACEP, CSS of the NAS/NRC for the FDAA of DoHUD.

Predicting the reaction of the public to an earthquake warning seems to be every bit as difficult as predicting the earthquake itself, and this, no doubt, contributes in great measure to the unmemorability of much of the report. No stone could be said to have been left unturned, even down to expressing concern that tourists be alerted and jewellers empty their shops. The consequences of a prediction could be enormous; evacuations, unemployment, political in-fighting, demands for more money for emergency services, nuclear reactor shutdowns, draining of dams, attempts to withdraw insurance cover, failure to keep up mortgage payments, political attacks on scientists, local/state/federal squabbles, profiteering in property sales, demolition of buildings. The consequence of error is appropriately large. Small wonder then that it has already been said by Garrett Hardin that prediction could be more devastating than the event itself. The committee's first

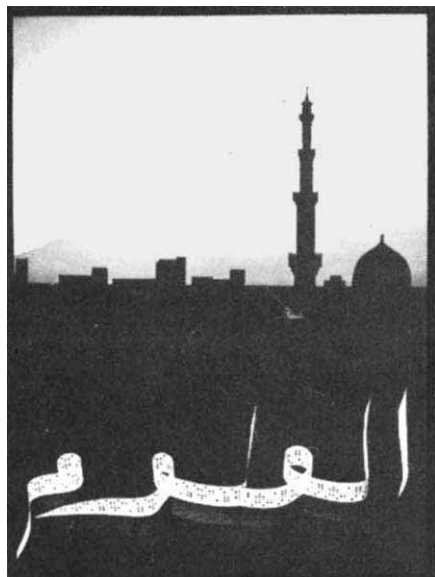
recommendation is that "The highest priority . . . should be . . . saving lives, with secondary attention to minimising social and economic disruption and property loss, *provided the costs of specific measures are within the limits that society is willing to accept*" (our italics). Quite apart from the vagueness introduced by the second half of the recommendation, it is entirely possible that the social and economic disruption and property loss from the prediction will greatly exceed that from the earthquake, and might counterbalance the benefit from lives saved.

The panel did not find this very acceptable; it was passed off as "a popular theme currently espoused among some scientists and science popularisers". Another idea that it didn't like was that scientists might desist from making public pronouncements, at least for a few years. "Earthquake prediction is a fact at the present time . . . attempts to suppress information concerning premonitory signs would certainly fail—as they should". Now this is half-truth. Many scientists with the best will in the world towards prediction are looking very carefully at the limited evidence available and still wondering not only how universally applicable predictive phenomena may turn out to be but also whether the quality of the data that will be accessible without an enormous investment of cash will be sufficient to give unambiguous warning in more than a very few zones. And which zones are to be favoured? There are, in California, as many major earthquakes off the San Andreas Fault as on it; no-one foresaw the San Fernando Valley as a danger zone in 1971. Earthquake prediction is a fact in much the same way that travel to the moon is a fact.

Further, it is only half-true to say, as does the report, that "there is no way to monopolise prediction capability" (and thus to prevent predictions being made). The tools required for prediction include access to a whole range of data libraries, and were the government to impose a moratorium on public announcements while seismologists were given time to conduct more thorough research, it is not at all clear that any independent agency would either wish or be able to jump the gun. There might be a general welcome in all communities, scientific, governmental, legal, insurance, constructional for a long pause before prediction was permitted. The spectacle that this report portrays is rather the opposite; of a well meaning panel riding a small band of experts as fast as possible towards a confrontation with nature.

On two counts, then, this report gives cause for concern: that scientists may be rushed, and that the case against making a major response to a prediction may not be adequately examined. And Turkey's misfortunes underline yet again how little even elementary information on earthquake proofing crosses national frontiers. □





Eden with oil wells

The stereotyped image of the oil-rich countries of the Arab Peninsula is that of an artificial, fragile, air-conditioned Eden made possible by the purchase of Western comforts, technology and scientific background. The picture holds an implied threat of collapse by the time oil reserves become depleted which, by most reckonings, should happen sometime during the next century, perhaps in the first half of it. That image is, however, only partly true. Large investments are being made in an attempt to achieve what should amount to a leap from the Middle Ages to the atomic era. On the Arab Peninsula, schools have blossomed and primary education has, in most cases, become compulsory. Technical training facilities have been established, medical schools are about to produce their first graduates and, in Saudi Arabia, one of the world's most modern specialist hospitals has opened its doors. Alexander Dorozynski reports on science and technology on the Arab Peninsula, where the avowed aim is to achieve scientific and technical self-reliance as rapidly as possible, and which, if the present effort is maintained, may well become the site of a unique achievement.

ALTHOUGH per capita income in several countries of the Arab Peninsula is among the highest in the world, income as a product of labour is among the lowest. Wealth has not had time to filter down to the majority of the population, and rich countries such as Saudi Arabia and Kuwait are still developing nations. But the means for rapid scientific and technological progress essential to development are available, and it seems that Arab leaders have understood the need to provide for future generations.

Thus, not only is there an effort to transfer Western technology, but to revive a scientific tradition that has largely been dormant since the days when the Arab empire extended from Spain to Samarkand, when cultured Arab envoys expressed surprise at the illiteracy of Charlemagne's court, and Arab scientists helped pave the way for the European Renaissance.

In Saudi Arabia, for example, illiteracy was widespread only 20 years ago, and the only centre of higher learning was the Islamic Studies Faculty, founded in Mecca in 1947. But since the University of Riyadh was founded in 1957 with a teaching staff of nine, the situation has changed rapidly. The College of Petroleum and Minerals (CPM) was set up with the help of the Arabian American Oil Company in 1963, and the Abd al-Aziz University was started in 1967 as a private institution sponsored by a group of business and political leaders.

Now Riyadh University has an enrolment of 6,000 students (about as much as the 100-year-old American University in Beirut) in eight faculties: arts, education, sciences, business, pharmacy, agriculture, engineering and medicine. The Faculty of Medicine is provided with professors, advisers and examiners by the University of London, and it will be the first faculty to move, in three or four years' time, to the \$500 million campus now being erected on the outskirts of the city. (In fact there will be two campuses, one for men, one for women.)

The Faculty of Sciences publishes a staff bulletin, whose contents reveal the wide range of interest and varying degrees of sophistication of research projects. A single issue, for example, contains a fairly simple and straightforward description of the morphology of the grasshopper and an analysis of climatic elements in the kingdom (with emphasis on potential agricultural developments), but also reports on subjects such as the electron spin resonance of several compounds. Professor A. A. Al-Khayal, Dean of the Faculty of Sciences, already voices a complaint familiar in academic circles, although unexpected in Saudi Arabia: lack of funds for facilities and

laboratory equipment.

The CPM, moving to an elegant, futuristic looking campus and chaired by Sheikh Ahmed Zaki Yamani, Minister of Petroleum and Minerals, teaches sciences, engineering science and applied engineering to some 1,000 students. High standards of admission are maintained by professors who, for the most part, come from American universities. The Abd al-Aziz University (arts, sciences, business and administration) has been integrated into the kingdom's educational system, and now has nearly 3,000 students.

These numbers alone, for a country of some 6 million people suddenly emerging into the modern world, represent an achievement. Another is the fact that women, traditionally kept outside the mainstream of life, now have access to primary and higher education. (This is attributed to the late King Faisal himself, and the influence of his wife, Iffat.) More than 200,000 girls, as well as half a million boys, attend school, and the numbers are constantly growing. Riyadh and Abd al-Aziz Universities have special teaching facilities for women. There is a shortage of women professors, and in many cases female students in separate quarters follow courses on closed-circuit television, and ask questions by telephone. Education at all levels (as well as medical services) is underwritten by the government.

Foreign observers familiar with Saudi institutions of learning generally agree that the level of standards is good and improving rapidly, particularly in professional education. But a straightforward comparison with Western institutions, which benefit from centuries of academic background and where religious and traditional values play a less important role, is difficult. Nevertheless, the admission of Saudi graduates as doctoral candidates at such institutions as MIT and Caltech is indicative of the standard that can be achieved.

An interesting (and controversial) initiative in Saudi Arabia has been the creation of the King Faisal Medical City and Specialist Hospital, which was inaugurated in April (before the graduation of the country's first crop of medical doctors). Conceived by King Faisal himself, the project was carried out under the leadership of Dr Rifat Alsayed Ali, personal physician to King Faisal and the royal family, who has coordinated the activities of dozens of foreign companies which have contributed to the building and equipping what is to become one of the world's most modern in-patient and out-patient specialist hospitals, offering facilities and skills seldom available under a single roof. The 250-bed hospital will be operated by an inter-

national staff of 750, part of which has already been contracted for. Future capacity will be 500 beds, with a staff of 1,200. Later this year, a clinical research department will be opened.

The idea, says Dr Ali, is not only to provide on-the-spot diagnostic and treatment facilities that many wealthy Arabs may seek in Boston, London or Paris, but to seek rapid medical progress in the region with the help of top foreign experts for whom the best possible working and living conditions are being provided.

The hospital has started operating on a limited scale, and on a paying basis. "Otherwise", says Dr Ali, "everyone will want to come here rather than to another hospital. But if any patient requires facilities and specialist treatment available only here, he will be admitted as a free patient if he cannot pay."

Another ambitious project in the kingdom is the development of agricultural research, considered as essential to self-reliance; the recently constructed laboratory of the Ministry of Agriculture near Riyadh is one of the most complete in the world in its design and equipment. It has yet to be fully staffed.

In Kuwait, where the development of a welfare state was started in 1951 by Sheik Abdallah as-Sabah and continued by his son and successor, Sheik Sabah as-Salim as-Sabah, schools have proliferated to provide for now compulsory primary education, and there is a splendid university (with a campus for women) which has research facilities with the latest equipment.

The progressive integration of women in a man's world is, here, more evident than in Saudi Arabia, where even secretaries are male. In Kuwait, women secretaries wearing modern dress are a common sight, and female researchers go unnoticed.

The Kuwait Institute for Science Research (KISR) has been operating for five years, and its prime purpose, in the words of its director, Mohammad Al Shamali, "is to build up a tradition for research. It is the first step, and perhaps the most difficult one to take."

While pursuing this goal, the KISR concentrates on research projects particularly suitable and useful to the country. Dr Al Shamali has long been interested by the potential use of indigenous desert plants, and a multidisciplinary research team led by Ibrahim Hamdan, an American-educated food scientist of Palestinian origin, is now trying to determine their nutritive value and their potential toxicity.

There are several hundred varieties of desert plant, perennial, annual and bushes, and little is known about them except that a little care, and a little more water, can greatly increase their

ONE of the most important catalysts to development in the Middle East is the Kuwait Fund for Arab Economic Development, created in 1961 as a government agency but granted complete independence in its finances, organisation, and policies. As Abdlatif Y. Al-Hamad, its Director General, likes to point out, one of its first principles is neutrality with regards to political and social systems adopted in the Arab countries. This neutrality seems to be more than simply token, as the fund has supported projects in countries with widely different political systems, such as Southern and Northern Yemen, Syria, Tunisia, Egypt and Mauritania.

Until last year, the Kuwait Fund functioned essentially as a regional development bank, providing assistance chiefly in the form of loans on concessionary terms, with interest rates around 3 or 4%, maturity about 20 years, and grace periods generally corresponding to the time needed for the completion of a project. (Some grants were also given, notably for technical assistance and the financing of technical or economic studies.)

In July 1974, the fund took a new turn when the National Assembly extended its operations to the rest of the developing world, and increased the declared capital from KD 200 million to KD 1,000 million, that is, more than \$3,000 million. Since then the fund has undertaken to support a number of development projects in Africa (livestock and sugar industry development in Uganda, river basin development in Senegal, tea plantations in Rwanda, textile spinning and weaving in Tanzania, construction of two harbours and a railway in Gabon.)

Other projects are being studied,

and the fund's director general has visited Asia, where several projects are expected to receive financial support (a urea plant in Sri Lanka, sugar factories and land reclamation in Afganistan, irrigation in Bangladesh, land rehabilitation in Malaysia).

In 1971 the Kuwait Fund was followed by the Arab Fund for Economic and Social Development, with headquarters also in Kuwait. The Arab Fund's charter restricts its activities to Arab countries, where joint developments are planned in such fields as highway transportation, telecommunication and agriculture. Finding that there is a shortage of well prepared national or regional projects, the Arab Fund has undertaken its own studies to help the development of a country in its regional context.

There doesn't appear to be an 'OPEC strategy' concerning aid to developing countries, but many OPEC members have reacted to the often-voiced charge that increased oil prices have crippled the development of poorer countries. Thus India has benefited from credit terms to obtain oil from Iran, Iraq and the United Arab Emirates, and for the poorer African countries, Arab oil producers have created a \$200 million fund to offset higher oil prices. Joint ventures have also been undertaken between rich and poor countries, for instance, between Kuwait and Mauritania to develop iron mines.

As there is little coordination between the various forms of aid financed by the OPEC, total figures are difficult to come by, but one estimate is that last year, more than \$5,000 million was committed to developing countries, either directly or through international agencies.

growth rate. Some of them have a good protein content, and, in a region where most of the fodder for cattle is now imported at considerable cost, they represent a potentially important natural resource.

A first step in the research project has been the identification of the principal varieties, some of which also exist in similar semi-arid regions of Australia, South and North America, but about which data is scattered or incomplete. This is now being followed by a determination of their nutritive value: a more sophisticated analysis of their carbohydrate, protein and crude fibre content. According to Ibrahim Hamdan, one perennial plant, atriplex, has been found to have a protein content of 16%, as high as that of alfalfa.

Many of the plants have been grown on the grounds or in greenhouses of

the KISR, and it has been shown that some of them may be suitable as a local substitute for more classical grazing plants that are costly to grow in the semi-arid tropics. Plants from regions with similar soil and climatic conditions have been included in the study, and attempts are being made to create a symbiosis between some desert bushes, annuals, and perennial plants (for instance, a deep-rooted shady bush, a climate-resistant perennial, and a succulent annual).

Recently, the cattle-feeding experiments were started on a desert plot of 20 square kilometres, acquired by the institute, and ways are being sought to complement this roughage with locally available additives, such as single-cell 'petroleum proteins' or microscopical algae (which are also experimentally grown at the KISR).

Toxicity studies have allowed the project to branch out into the study of the potential medicinal properties of some of the plants. Ali Anani, a chemist, has identified a number of alkaloids and other substances that are being studied in the particular context of traditional Kuwait medicine, which itself is probably an offshoot of ancient Arabic medicine, still practised by healers (*attarin*) who coexist in Kuwait with the free, Western-style medical services provided by the state. The *attarin* use herbal medicines, but jealously guard their secrets, sometimes mixing a number of substances in their preparations, apparently to camouflage the active ingredients. Some of the plants have already been shown to possess antibacterial activity. Soil bacteria and fungi have been included in the study, as well as hormone-like promoters of plant growth and soil enzymes that could be used to improve fibre digestibility.

Dr Mohammad El Shamali and Dr Hamdan believe this research may well turn out to be of significant importance in the development of agriculture, but also that it is a good way to train researchers of varied disciplines and nationalities to work together as a team.

It would be meaningless, again, to compare research undertaken in Kuwait with that of an MIT, Rockefeller or Pasteur Institute. But the effort is obvious, and the results, considering the lack of research background and tradition, encouraging indeed.

The pattern is repeated elsewhere on the peninsula. In the small state of Bahrain (where oil was found in the early 1930s) there are more than 100 schools for a population of 220,000, and several vocational training centres. In tiny Qatar, there is a technical school, a school of commerce, a teacher's college, and a total of about 90 schools; last year, the ratio of teachers to the population was about 1 to 1,000.

At the same time, the 'technological transfer' continues, with an increasing amount of goods being produced on the spot (albeit with the help of foreign technicians) rather than imported. Thus, while a few years ago the story was that a desert prince would discard his Cadillac when the ashtray was full or the tank empty, now there is a plant in Saudi Arabia where cars are overhauled and rebuilt, and arrangements are under way to set up assembly plants. In Riyadh, production of pharmaceuticals is expected to start this year; the General Petroleum and Mineral Organisation (Petromin), a public corporation owned by the state, runs a steel rolling mill on the Red Sea coast south of Jiddah. Local

THE government of Saudi Arabia is planning to spend \$12,000 million of its oil wealth in an effort to turn the country's desert green.

The desert lies 180 miles east of Riyadh and is the centre of a massive desert reclamation programme, part of a \$143.5 thousand million five-year development plan.

Altogether, the Saudi Arabian government plans to turn 4.18 million hectares of sand into farmland, which according to the Under Secretary of Agriculture, Mr Taher Efeid, will take at least half a century to accomplish.

At present, only 1.4 million acres of farmland are cultivated in the desert monarchy. This earns more than \$25,000 million in revenues annually.

The Kingdom envisages improving and regulating underground water resources and installing an efficient drainage network to reduce salinity in various areas.

Five research centres, dealing with fishing, insecticides, fodder, seeds, fertiliser and poultry have been set up in Jeddah, Riyadh, Jassa, Hofuf and Qatif. These centres also operate model farms.

Two hundred and fifty types of rice are being tested to cultivate 167,000 acres which will raise the production from 75,000 tons to 100,000 tons over four years.

To encourage private investment in agriculture, the government will distribute reclaimed land at an average of 32-160 acres per farmer. The farmers will be given three years to prove they are capable of tending the land satisfactorily. Otherwise, the land will be taken and given to

another more experienced farmer.

In the incentive programme, farmers will be able to purchase agricultural machinery at 55% of regular prices.

The prices of imported fertilisers will be reduced by 50% and dairy equipment will be sold at 70% of their import prices.

Farmers will receive \$2.50 annually for each sheep and \$14 a year for every she-camel.

There are 17 medium-sized dams in the country and it is hoped gradually to increase these to 23. Most of the dams control rain waters in the southern region of Jaizan and Abha.

The biggest—Jaizan dam—is approximately 1,000 feet long and 125 feet high with a capacity to store enough water to irrigate 50,000 acres. It cost \$27 million to build.

Seventy million dollars were spent on the Hassa irrigation and drainage programme which increased the cultivable area around Hofuf from 20,000 acres to 50,000 acres.

Also under the plan \$51.8 thousand million was budgeted for electrification projects, desalinated water production is expected to rise from 57 million gallons a day to 163 million and a total of 270,000 new homes—some in reclaimed areas, are to be built.

Agriculture officials say that 'the days of scratching a living from small sandy plots are over for the farmers and Bedouins of Saudi Arabia'.

Vegetable production is near self-sufficiency, reports say, and the farmers who had deserted their salinity stricken farms are now returning.—*Gulf Mirror*.

cement factories supply about half of the demand of a booming construction industry; fertilisers and polyethylene bags to store them in are made on the spot.

In Bahrein, aluminium ingots are made from imported bauxite and exported. Kuwait has an aluminium smelter, clothing and household goods factories, plants making prefabricated houses and building materials. Research in hydroponics has been going on for several years, and some vegetables produced in this way now reach the market in Kuwait city.

Although one does not find on the peninsula the relatively sophisticated native technology such as is frequent in Beirut, Amman or Cairo, it should be remembered that only a few years ago, the Arab Peninsula was quite literally a scientific and technological desert. An added impetus to this awakening comes from collaboration with the poorer Arabs—Egyptians, Jordanians, Lebanese, Palestinians—

and an effort is being made to attract from Europe and particularly from the United States Arab scientists who have become established abroad, and even taken foreign nationalities. Most come on short-term contracts (a sabbatical year for instance) but a few find their ancestral land so transformed that they are tempted to remain.

There is, understandably, a problem posed by instant wealth. Although it has certainly given a boost to this 'leap' into the modern age; sometimes it becomes an obstacle by removing some of the incentive to learn in a system where not only is there no tuition but nationals receive a substantial living allowance. It seems, though, that there is a consciousness of this, and in several centres of higher learning, incentive have been set up to reward effort and achievement. Talented young Arab graduates are subsidised to continue their studies abroad, and are assured of finding a rewarding position when they return home. □

international news

MEMBERS of the House of Representatives returned to Washington from their summer vacations last week and promptly passed a bill which, according to its supporters, may save the United States as much petroleum as the entire supply from Alaska's North Slope. The key to that amazing prediction is the successful development and mass production of an electrically powered car—an idea which has been backed in recent years by plenty of rhetoric but few dollars.

The bill would provide as much as \$160 million over the next five years for a research and development effort designed to improve battery technology and to pave the way for commercial introduction of electric vehicles on a grand scale. That amount should be compared with the trifling sum of \$1.166 million which was spent by the federal government on battery-powered vehicle studies between 1969 and 1974.

Battery-powered cars are, of course, already in commercial production, but their range is limited to about 50 miles because they use conventional lead-acid batteries which need frequent recharging. Nevertheless, as one proud owner of an electric vehicle, former Atomic Energy Commissioner Clarence E. Larson, pointed out in a letter to the *Washington Post* last week, 50 miles is quite far enough for most city trips.

The potential market for electric cars is certainly large. For one thing, it is estimated that 50% of all car journeys in the United States are less than five miles, which says a lot about unnecessary use of the automobile. A more pertinent statistic is that about 98% of all trips taken with second family cars, lie within the 50-mile range of present battery technology. The second car, which is part of the American way of life, is therefore clearly the target of the electric car industry if, or when, it is in business.

Other advantages claimed for electric cars are that since they will mostly be charged up overnight, when electricity demand is low, no new electricity generating capacity will be required. It also goes without saying that the lack of pollution from the car itself will be a good selling point. And much is also made of the fact that transportation now accounts directly for about 25% of total energy use in the United States; the potential saving in imported oil by replacing petrol-driven cars with electric vehicles is

Congress boosts electric car research

by Colin Norman, Washington

therefore immense. (But it should be noted that total energy savings will be negligible since the energy required to produce the electricity will offset the savings in petroleum. The important point, however, is that the net effect will be to shift energy consumption away from oil to coal and nuclear power.)

But the picture is not quite as bright as the most ardent enthusiasts maintain. Political, technical and economic problems could all stunt the growth of the fledgling electric car industry.

First, the costs. A study carried out by the Environmental Protection Agency, which was published last October, concluded that electric cars would be between 20 and 60% more expensive overall than equivalent conventional cars until battery develop-

ment significantly reduces battery depreciation costs. The expense of charging up the battery is only a part of the total operating costs, and some technological advances will be required both to extend the capacity of batteries and to make them more durable.

As for the range of electric cars—probably the chief commercial drawback of present models—a committee of the National Academy of Sciences estimated in 1973 that it would take at least four years, assuming adequate funding, to develop an advanced battery power plant with a range of over 100 miles. The committee estimated that it would then take an additional seven to 10 years to mass produce cars with advanced batteries. Those time scales are now considered to be optimistic, however, largely because private and public funding has not built up as rapidly as expected.

Finally, neither the automobile industry nor the oil industry have shown much enthusiasm for the electric car. Both are powerful lobbies and according to some observers they have been effective in holding up the development of battery technologies. Whatever the truth of allegations, it would certainly be a major operation for Detroit to

Pasteur Institute on way back to solvency

AFTER reviewing a report of the Pasteur Institute's financial status, Madame Simone Veil, Minister of Health, has persuaded the French government to increase credits allocated to it from some 20 million francs in 1975 to 50 million francs in 1976. This is in addition to subsidies to the institute's teaching activities, and to total or partial salaries paid to some Institute researchers by the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale.

The new budget is to be approved by the parliament, but it has already been announced by the Ministry of Health and, barring the unforeseen, should be passed.

According to Dr Joel de Rosnay, director of development, the grant will ensure solvency, and enable the institute to continue its development following a businesslike reorganisation earlier this year. It covers about half of the expenses of the foundation, the research branch of the institute. The

rest will come from other resources, including interest on capital, donations, and income earned by Institut Pasteur Productions, the institute's commercial arm. (A forthcoming innovation will be the standardisation of the "mutatest" devised by Dr Bruce Ames of the University of California at Berkeley. The test relies on the ability of carcinogenic chemicals to cause mutations of strains of the common bacteria *Salmonella typhimurium*. Developed in France in collaboration with the International Agency for Research on Cancer in Lyon, it will be available commercially).

In addition, during the recent visit to France of Sheikh Sabas Al Salem Al Sabah, the Pasteur Institute received from Kuwait a donation of 5 million francs, about half of the institute's annual deficit. This donation will be used "out of budget" to finance research on infectious and tropical diseases, embryology and cancer.

—Alexander Dorozynski

FOUR years after its creation, the Canadian Ministry of Science and Technology is to be reorganised. News of the change was given by the Minister of State for Science and Technology, Mr C. M. Drury, at the thirteenth Pacific Science Congress. "The ministry's job has been to advise the government on how science and technology can be used and developed to the best advantage of Canada", he said. "This is a difficult task and we have learned much about the capabilities of such a horizontal ministry in the four years of its existence. Based on this experience the ministry is now being re-organised in order to enable it to function more effectively."

It will be organised at the working level to communicate with the three principal segments of the scientific community—universities, industry and government and will include the many professional and learned societies. The ministry's policy-making role was defined by Mr Drury in two categories: major continuous policy assignments with long-term implications, such as development of a national capacity to study and assess the impact of science and technology on society; and development of policies and programs for the co-ordinated and large-scale use of scientific resources beyond the scope of a single agency or government, such as the oceans policy.

"The job of the ministry", said the minister, "is not to displace or duplicate the roles of existing departments and agencies in science, engineering and technology. Nor will we try to become a sort of national oracle for science, issuing decrees on what scientists in government should and should not be doing."

● A discussion on Canadian science policy showed how far apart the bureaucrats and ordinary scientists are. While the Minister outlined precisely what science policy meant to the government, the Dean of the University of British Columbia's Faculty of Graduate Studies, P. A. Larkin, declared himself confused and be-

wildered by the concept itself—and abashed by the way in which it was being carried out. And as for communication on the subject between scientists and government, he said: "You have about as much chance of getting through as if you were reciting Gaelic poetry to a deaf seagull."

Larkin called his address "Ask Archimedes", because, he said, interest in science policy goes back a long way: "For example, Archimedes used his talents in many ways in the service of his native city of Syracuse, particularly in times of war, and presumably the king of Syracuse thus had a 'science

Science in Canada

from David Spurgeon, Ottawa

policy'—which was, 'Ask Archimedes'."

On the other hand, said Larkin, Canada's science policy for 50 years was "me too", meaning that it simply copied the policies of other countries. "And its implementation was accomplished when C. D. Howe, the 'minister of everything', played golf with C. J. Mackenzie, who, as the President of the National Research Council, was the architect of the national determination to build scientific intellectual resources."

Later, Larkin continued, many reports were published on the national scientific effort. "Virtually every report by a committee or task force on science policy in the last 20 years has had something to say about the failures in coordination, communication, and integration; and the frightful sins of duplication, procrastination, and bureaucratisation. Since the most recent reports sound very like the older ones, it seems likely that either no one reads the reports or, if they do, they pay little attention."

Outlining some of the main thrusts of science policy thinking, Larkin declared himself—and others—be-

wildered. It has become obvious that so many changes are taking place so rapidly, he said, that no one has a good grasp of where we are collectively headed. "Even if someone could see it all clearly, it is most unlikely that he could do anything about it. World wide, science and technology are in a state of extremely rapid and turbulent evolution. There are so many scientists, and communications have become so good, that problems are perceived, attacked, solved and the solutions applied almost before most of us are aware of them. . . . Science policy has become a curiously all-pervasive and eclectic exercise in which it is increasingly difficult to judge whether you are worrying about the right thing."

Furthermore, said Larkin, most reports concerned with science policy are obsolete by the time they are published. Few read them any more. Most are "essentially a condensed summary of a consensus that has not only been reached but partly implemented before the report is made public. With several such studies going on at once, the national capital often gives the impression of being a full time continuous scientific conference in which everybody is writing papers and no one is attending the sessions at which the papers are presented."

The committee, task forces, commissions and the senior Civil Service, said Larkin, "move along close to the margin of what almost seems uncontrollable change, documenting and formalising what has already happened, arguing a bit about who thought of it first (when none of them did), performing the apparently essential tasks of administering and reorganising, but in reality never realising they are like the bubbles in beer, produced by pressures that are quite incomprehensible."

Mr Drury called Larkin's comments about communication between scientists and government "cynical", and said that some institutions and mechanisms already existed for such communication, and that one task of his ministry was to devise other methods.

switch some of its production to an entirely new automobile concept. The big car makers have, after all, raised enough complaints about the relatively minor production changes necessary to meet air pollution standards.

Those obstacles to commercial production are the chief factors which prompted the House of Representatives to approve massive federal support for a research and development effort last week. The bill, which was largely the work of Representative Mike McCormack, instructs the Energy Research and Development Administration to

devote more money to developing advanced battery technology. But the heart of the measure is a demonstration programme through which the federal government will underwrite the costs of at least 7,500 electric cars during the next five years.

The purpose of the demonstration programme, according to a report written by the House Science and Technology Committee, "is to get present and future state-of-the-art electric vehicles out into every region of the country". They will be used in federal, state and local government fleets, by

businesses and by individuals, to gain experience of driving performance, maintenance costs and so on. An important aspect of the operation, however, is that it will provide an incentive for industry to set up the production facilities, which should pave the way for mass production later.

The bill was passed easily by the House—by 308 votes to 60—and the Senate Commerce Committee will hold hearings on a similar measure early next month.

It is expected that the Senate will also pass it easily. □

correspondence

Good for science?

SIR,—Your editorial "Good for people, bad for science" (July 10) requires a prompt comment, for you apparently advocate a major change in the system of academic tenure. At the same time, your editorial seems to indicate neither an awareness of the complexity of the issue, nor of experience with alternative tenure systems, nor of the considerable literature in this area; I refer here primarily to the tenure system that is very widely used in the USA, the role of the American Association of University Professors (AAUP), and the many articles and books in which this subject has been discussed.

Tenure is generally awarded, in US colleges and universities, after a probationary period that should be long enough to provide sufficient time for a reliable assessment of the capabilities and promise of a faculty member, yet should not be so short as to lead to an unacceptable proportion of bad decisions. The AAUP guideline of a maximum probationary period of seven years is widely followed, but universities will often award tenure much earlier in an attempt to hold on to younger people of exceptional ability. This does not, of course, ensure that all those who have tenure will continue to merit it, and removal for cause (including demonstrated incompetence, as decided by an elected faculty committee) is recognised, though rare.

The major benefit conferred by tenure is the freedom that faculty members should then have to study, discuss and explore without fear, views and topics that may be highly unpopular or controversial at that time. Most scientists pursue careers that are models of conformity and caution, but some may wish to be more adventurous. Research involving human subjects raises strong passions—consider the continuing debates over foetal research and genetic engineering. If these scientists were subject to renewable contracts, it is safe to predict that, for many, caution would increase as the renewal date approached, and topics that may seem of major scientific interest would be neglected if adequate protection could not be guaranteed for those who wished to work on them. These are not hypothetical fears of imaginary but unlikely situations: these pressures on the academic community exist and should be recognised.

There is another serious objection to the proposal for contracts: who would review them and make the decisions on renewal? A senior administrative officer in the university or in Whitehall? With what advice? Or a group of faculty members or the department chairman? One may reasonably claim that professional competence can only be judged by others in the same discipline, but this is more likely to lead to leniency on the part of the reviewers in the hope of similar treatment when their own renewals come up. Leaving the decision to a department head carries other risks: renewal will provide an excellent opportunity to get rid of the abrasive members of the faculty. Indeed, extensive AAUP experience has shown that a major proportion of the threats to faculty members come from other faculty members and from department heads and deans who were once also faculty members. Overall, the apparently attractive idea of renewable contracts bristles with severe problems of implementation, and should not be lightly suggested without at the same time treating this side of the suggestion.

It is not only on the frontiers of research that feelings become inflamed and faculty positions are threatened. Some of us hope that more university scientists will become engaged in what Ravetz has termed 'critical science' (*Scientific Knowledge and its Social Problems*, Oxford University Press, 1971). This involvement in current issues should not need to be partisan but can be in the direction of elucidating complex technical issues to a broader public. Here again, these scientists need the protection of tenure, for, it is clear, those employed in industrial and government laboratories do not have the freedom to engage in these issues unless they are generally on the side of their sponsors. University science departments will probably provide virtually the only source of independent comment, and these scientists will not be able to play a responsible role if their jobs are in jeopardy. Here again, this is not some hypothetical possibility: as 'critical' science has slowly developed in the USA, government and industrial scientists have been conspicuous by their silence.

Those of us who have had experience in faculty matters, with tenure and with the AAUP, recognise that the weakness of the tenure system is the

protection it extends to those who prefer to relax and do little or nothing, but most of us consider this is a modest price to pay for the many advantages. A tenure system can operate no better than those who have to make the decisions; some decisions will be good and others bad. A recent thorough examination of the tenure system (*Faculty Tenure*, Commission on Academic Tenure; W. R. Keast, chairman; Jossey-Bass Publishers, 1973) produced a strong endorsement.

In summary, then, your editorial is disappointing. Perhaps the British universities' tenure system does need overhaul; perhaps a probationary period similar to that in the USA should be considered, along with a greater involvement of the non-professional faculty in matters of faculty promotion and tenure. But I would hope that notice is taken of the extensive and documented experience before adopting a contract system that has a superficial appeal but is potentially severely detrimental to academic freedom.

Yours faithfully,

M. W. FRIEDLANDER

Washington University,
St Louis, Missouri 63130

Fellows overseas

SIR,—I can well understand the frustration felt by UK postdoctoral fellows overseas as described by Richard James (August 7). Since I doubt that the "structure of the UK University system" will change, I can perhaps do a little to put the position straight, at least as I see it from my department, and make a suggestion.

All of us want very much to appoint the best people to the few lectureships available. Our system of advertising the posts as soon as we are assured of the finance is not only efficient but is fair and should not be classified as ponderous. It would unnecessarily delay matters to set a deadline for receipt of applications which gave time for the advertisements to be seen by all potential applicants overseas. In my experience we never keep strictly to the deadline, and will always consider late applicants and give special consideration to those who are overseas. A brief airmail statement to the effect that a formal application is on the way is certainly helpful. In short I do not believe that the heads of departments handling the regrettably

small number of lectureships are unsympathetic and ponderous.

I agree that it would be better if we could all plan appointments much further ahead than we do at present. I think this is unlikely to happen in the foreseeable future. May I suggest, therefore, that it would be very helpful if overseas postdoctorals who are interested in returning here keep in touch with their former head of department and, more important, make an arrangement with a former colleague who would provide information concerning any vacancy that might be of interest.

Yours faithfully,

P. N. CAMPBELL

Department of Biochemistry,
University of Leeds, UK

Engineering standards

SIR,—In "Round Britain (July 31) you report, and seem to approve, the Committee of Vice-Chancellors and Principals' pressure to increase the academic standard of British engineers by encouraging employers to insist on higher degrees. My job is engineering design and project management in an advanced field (fast reactors), and I can sympathise with the CERN engineers who despair at the often woefully inadequate capabilities of British industry; but I should need a lot of convincing that this has anything to do with the number of years people spend at university. Most would agree that where fast reactors are concerned the ranking order of national success is France, USSR, United Kingdom, with USA, Japan and West Germany following somewhere. I am pretty certain that this does not correlate well with the national proportions of engineers holding higher degrees, but perhaps the vice-chancellors would like to give us some statistics?

Success in advanced engineering projects obviously demands a reasonable level of theoretical ability, but it depends much more on the attitudes and policies prevalent nationally and within industrial firms, and on the ability of engineers to handle unfamiliar and very complex technical and organisational problems with imagination and good judgement. I suggest that to encourage large numbers of men to spend two or three years calmly researching esoteric problems is not likely either to improve government and public attitudes to engineering or to develop the necessary flexibility in the men concerned.

For my money I would support the Open University against all the dignity of the vice-chancellors; its technology courses are designed to stimulate thoughtful, imaginative and socially responsible attitudes. It is being squeezed financially by the government

that conceived it, and it is also not yet receiving the recognition it deserves from professional bodies. In these circumstances to channel extra resources into expanding post-graduate engineering departments would be short sighted indeed; what is needed in British engineering is more excellence and public acceptance at first degree level, not more narrow specialisation.

Yours faithfully,

JOHN A. GATLEY

Knutsford,
Cheshire, UK

All at sea

SIR,—Recently (May 8) you reported on some of our work in your editorial "For those in peril on the sea". Scientists and crew aboard research vessels co-exist under tension, created mostly by the needs of scientists and felt mainly by crew; tension which can and does erupt into unpleasantness. This costly problem is now recognised by the Canadian and German governments, for example.

Our most basic finding is that this situation results from locking two warring sub-cultures—academicians and people of working-class orientation—on a ship at sea where they cannot avoid each other as on land. The single most important cause of tension is the "data hunger" of scientists and their degree of sensitivity to the relaxation needs of the crew.

Many scientists, yourself included, have criticised our findings. Unfortunately, they all did so having read only a news release (which mentioned only Bernard). Published work should be read before criticism. We question your saying, "for half the price of placing an anthropologist . . . (you) would have been happy to tell" ONR about shipboard life. The total cost was less than three days' 1972 ship time (and covered part of a larger project to describe human relations numerically).

It is important for scientists to realise that mariners' views differ intrinsically from your editorial. For example, you compare the chief scientist with a referee, and believe that captains always want to get ships to port a day early. But captains also see themselves as referees, and believe that scientists always want to get ships to port a day late. Whether any of these sentiments are true (in fact ships do dock both early and late) is irrelevant. The important thing is that these sentiments are believed by those concerned, and insensitivity by others to these beliefs causes conflict.

Anthropologists rarely learn much that the natives, like yourself, do not know. Your observations affirm some of our work. We hope to hear from

other members of the ocean science community on conditions aboard any nation's vessels. A dialogue might help prevent Bransfield-like incidents.

PETER D. KILLWORTH

Cambridge, UK



RESULTS of competition No. 1. Readers were invited to supply the minutes of an illicit seminar held by scientists somewhere in the Western world. Though we withheld judgement for as long as possible, we were finally forced to recognise that there simply wasn't a welter of witty replies held up in the post somewhere. It may have been that the subject was too baffling for many to cut their teeth on. Winner from the half a dozen entries was E. Jarvis, of Clapham, London (entry below). A further prize goes to Scott Gilbert, of Johns Hopkins University, Baltimore, for a near miss.

AMSS in session

THE Anti-Metric Society of Scientists held a meeting at a secret location in Mile End Road.

It was resolved that they would not budge an inch in their efforts to resist metrication and that they would continue to fathom out ways to circumvent its introduction. The problems were more than pint-sized but there was still much mileage in their opposition activities.

Contingency plans were drawn up to ensure that members would at least obtain their pound of flesh; an issue would be made of diamond cutters for reducing litre glasses to pints and pocket saws for cutting down metre rules to yards.

The ladies' committee announced that Valentine Cards inscribed 'I love you a bushel and a peck' would be available to members by the dozen.

Competition No. 2. An easier test this time, with a longer time limit of six weeks to allow for mental as well as postal blockages. A prize of £10 awaits the winner (or winners):

There was a young lady called Bright,
Who travelled much faster than light,
She went out one day

In a relative way,
And arrived home the previous night.

Ms Bright and her épéeist companion Fisk are already immortalised in limericks with a scientific flavour. Competitors are asked to submit further examples based in similar vein, on fundamental scientific principles, observations and so on. □

news and views

A YEAR ago *Nature* published a communication by J. B. Barbour (*Nature*, **249**, 328; 1974) on "Relative-distance Machian theories". There were some general comments in *News and Views* (*Nature*, **249**, 305; 1974), but no discussion of details because Barbour promised another model having 'more interesting properties'. This was recently published under the title "Forceless Machian dynamics" (Barbour, *Nuovo Cim.*, **26B**, 16; 1975). It may be useful now to comment on the model without again reflecting upon wider aspects of so-called Machian concepts. This is possible only with sufficient indication of Barbour's mathematical procedure, while keeping it as concise as possible; I concentrate on his recent paper, but rely upon the earlier one for certain explanations.

Barbour considers a system of N particles of mass $m_1, \dots, m_i, \dots, m_N$, with $\sum m_i = M$, where m_i is an intrinsic number associated with particle i . They are in a three-dimensional Euclidean space; any configuration of the system may be specified by a point in $3N$ -dimensional space (since only relative distances are to be significant) called relative configuration space (RCS). The history of the system is a curve in RCS; a suitable parameter along the curve defines time t . Barbour assumes that the history is got by making a Lagrangian function L extremal, and a necessary consequence of his interpretation of Machian requirements is that L is a function only of the distances r_{ij} between the pairs of particles and their t derivatives. For the system of simple particles he postulates

$$L^2 = \sum m_i m_j \dot{r}_{ij}^2 / r_{ij}$$

Now, however, Barbour is interested in replacing these particles by bodies admitting some internal motion. In order to disclose the consequent possibilities, he constructs a highly particular model; it is probably the simplest that is capable of exhibiting the features to be discussed—it is certainly ingenious.

For simplicity, the model is confined to a Euclidean plane; as before, it consists of a number of masses, but here each mass m_i is a ring of radius a and uniform line density ρ_i where $m_i = 2\pi a \rho_i$ and $a \ll r_{ij}$ for all i, j ($i \neq j$) so that r_{ij} may still be called the distance between bodies i, j . The angular coordinate corresponding to rotation of ring i about its centre is ϕ_i .

As he says, Barbour proceeds to calculate a test-particle 'situation'—

Mach in detail

from W. H. McCrea

'scenario' in the vernacular. He considers a test-ring, also of radius a , of mass $m \ll m_i$, and with angular coordinate ϕ , where the rotational velocity $\dot{\phi} = a\dot{\phi}$ and the translational velocity of the test-ring will be found to vary with its location relative to the others, but m is so small that its effect upon the motion of the others is negligible.

At time t the test ring has polar coordinates r, θ relative to some particular ring S of mass m_s , which is assumed to rotate at constant speed $a\dot{\phi}_s = c$. Then if the appropriate part of the foregoing Lagrangian is expressed in terms of elements $ap d\phi$, $ap_s d\phi_s$ of the rings m , m_s and integrated over them, they make a contribution to L^2 , up to terms in $(a/r)^2$,

$$\{ \frac{1}{2} m m_s (2\dot{r}^2 + \dot{\chi}^2 + c^2) \} / r$$

For the model, Barbour further assumes all the numerous remaining rings to be distributed uniformly round a circle of radius $R (\gg r)$ with centre at S , the rings being at relative rest but all having the same rotation $a\dot{\phi}_i = c$. He finds these contribute to L^2 , to the appropriate approximation, the amount

$$\{ \frac{1}{2} M M (\dot{r}^2 + r^2 \dot{\theta}^2 + \dot{\chi}^2 + c^2) \} / R$$

According to his postulates, the motion of the test ring is obtained by treating the sum of the two contributions as the effective Lagrangian $\tilde{F}$, dividing for convenience by the constant M/R . For a well known property allows us to use $\tilde{F}$ instead of its square root, provided the variable t has been suitably normalised. The Lagrange equation in χ then gives

$$\partial \tilde{F} / \partial \dot{\chi} = \dot{\chi} + B\chi = K$$

where $B = Rm_s/rM$, and K is a constant.

At this point Barbour assumes $K=c$, and he takes $B \ll 1$ as it is in his application. Then by further appeal to standard theory of variational principles, he shows an equivalent Lagrangian $\tilde{F}$ for r, θ to be, to the required approximation

$$\tilde{F} = (\dot{r}^2/2) + (r^2 \dot{\theta}^2/2) + (Rc^2 m_s / Mr) + (Rm_s \dot{r}^2 / Mr) - c^2 (Rm_s / Mr)^2$$

In order to give the model a tentative

physical meaning, Barbour identifies the particular ring S with the Sun, the test-ring with a (small) planet, the distant rings with the rest of the universe. He can then identify R , M with the conventional radius and mass of the universe, and then he quotes the 'cosmic coincidence' that the Newtonian cosmological constant $G \approx Rc^2/M$, provided c be taken as the speed of light. Then in $\tilde{F}$ the first two terms are the inertia terms and the third the gravitation term in the Lagrangian for a particle moving in the field of a mass m_s according to Newtonian mechanics and gravitation. So Barbour considers he is on the track of an explanation of inertia and gravity "in terms of the single simple concept of mass in relative motion" and that this "hinges on the existence of the conjectured internal motion." The latter he would naturally expect to be more complicated in a more realistic model. He goes on to speculate on the possibility that "the rest-mass energy of the particles is associated with an incessant internal motion at velocity c ", so that the internal motion would account for both inertial and gravitational mass. He hopes for a generalisation of his Lagrangian that could be regarded as comprehending special relativity and quantum mechanics.

The last two terms in $\tilde{F}$ are what Barbour calls (small) Machian terms. He states that the corresponding terms in the analogous three-dimensional model yield one-sixth of the Einstein value for the advance of the perihelion of a planet. Presumably he hopes for a better value in a more developed treatment, and he mentions several other expected developments. Lastly he suggests that his treatment may be regarded as a realisation of a system of forceless dynamics envisaged by Hertz but never constructed.

It is easy to ask several questions: Can the treatment be applied to other obvious problems? What is the status of the geometry and of time in the theory? What is the justification for the use of a variational principle and for the Lagrangian employed? Has it a meaning to say that all energy may be kinetic since, if there is no rest mass, we ask what carries the kinetic energy? Also there are questions of detail: For example, why does Barbour identify his constant K , which is a constant of a particular motion, with c which appears as a universal constant in his scheme?

Nevertheless, at this stage the work is clearly exploratory and one should ask rather what promising positive indications are emerging. It is certainly so

interesting that one must wait at least to wait and see what Barbour will do next. It might prove to be just another way of developing general relativity, or it might yield general relativity with certain constraints that render it Machian in

some sense in which general relativity is not necessarily Machian. Or, as Barbour seems chiefly to hope, it may give something like Machian relativity incorporating some sort of quantum mechanics. □

layer and gradually die. From these observations, Rezai and Yoon suggested that the massive cell death is a consequence of the failure to migrate.

This conclusion was supported by subsequent investigations. In a series of electron microscopic studies, Rakic and Sidman (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 240; 1973; *J. comp. Neurol.*, **152**, 103, 133; 1973) noted that the disorder is accompanied by defective Bergmann glial fibres. It had been shown previously that these glial fibres guide the migration of the granule cells into the granular layer (Rakic, *J. comp. Neurol.*, **141**, 283; 1971). Early in development, before the granule cells have divided, numerous glial cells extend their Bergmann fibres radially towards the external granular layer. The postmitotic granule cell then attaches itself to the endfeet of the glial fibre to descend to the inner region of the cerebellum, while undergoing an ordered series of transformations in shape as it migrates. As the cell body descends, it leaves a T-shaped axon behind, which is to become a parallel fibre. Thus, in order to move across a complex terrain that changes in composition with time, migrating granule cells use the Bergmann fibre as a sliding ladder. Rakic and Sidman believe that the target of the weaver gene mutation is the Bergmann glial abnormality which causes, and precedes, failure to migrate and the death of granule cells. As evidence for this assertion, they note that the glial abnormality can be detected long before the

Synaptic remodelling in the mutant cerebellum

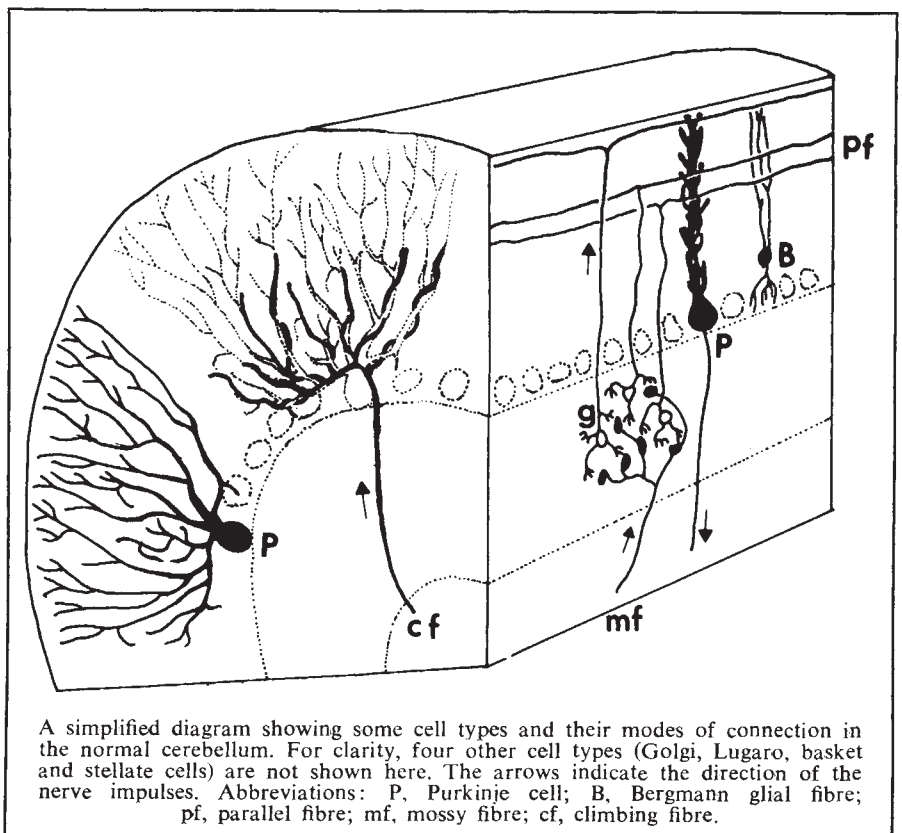
from Shin-Ho Chung

OVER the past few years, anatomical studies on neurological mutant mice have shed new light on the mechanisms governing the formation of the mammalian nervous system. In particular, the cerebellar cortex of these mutants has proved to be fertile ground for investigating certain fundamental questions about neural development. This is because the cell types and their interrelationships, along with the normal sequence of developmental processes, are better understood in the cerebellum than in most other parts of the nervous system. Because a single gene mutation often interferes with developmental processes in a precise and specific way, examination of the affected brain will reveal the primary target of the mutation as well as the consequences of such a previous mistake on the subsequent formation of the brain. A recent ultrastructural study on the 'weaver' cerebellum by Sotelo (*Brain Res.*, **94**, 19-44; 1975) is aimed at elucidating these questions.

The cerebellum of the normal mouse contains six types of neurones distributed in three layers; these are, from the outside in: the molecular layer, the Purkinje cell layer and the granular layer. The cells interconnect in regular patterns and integrate the inputs conveyed from other parts of the brain by two main afferent fibre systems. The Purkinje cell bodies which lie in a single row just above the granular layer, extend their flattened dendritic trees towards the surface of the cerebellum. Climbing fibres, one of the two main inputs to the cerebellum, form numerous synaptic contacts with the shafts of these Purkinje cell dendritic trees (see figure). The second input (mossy fibres) influence the Purkinje cell mainly through granule cells. Profuse branches of the granule cell dendrites curve around and encapsulate the expanded terminal of a mossy fibre, forming a tangled ball of dendrites and axons. Axons of the granule cells, known as parallel fibres, are extended towards the molecular layer, where they bifurcate, and running perpendicular to the Purkinje cell dendrites,

form numerous synapses with their spiny branchlets.

When this intricate structure is severely perturbed, as in the homozygous weaver mouse, the animal exhibits behavioural abnormalities characterised by tremor, hypotonia and ataxia. The autosomal, recessive weaver gene mutation arose spontaneously, and subsequent histological examination of the affected animal revealed that almost the entire population of granule cells is missing (Lane, *Mouse News Lett.*, **30**, 32; 1964). During postnatal development of the normal mouse, postmitotic granule cells migrate down from the germinal layer (near the surface of the cerebellum) through the Purkinje cell layer to form the granular layer. Rezai and Yoon (*Devl Biol.*, **29**, 17; 1972) first showed in the homozygous weaver mutant that granule cells proliferate normally, but remain at the germinal



appearance of granule cells, and that migration is slowed along the abnormal glial fibres in heterozygotes. Moreover, morphological examinations show no difference in the granule cells of normal and weaver mice before they start to migrate. Sotelo and Changeux (*Brain Res.*, **77**, 484; 1974), however, dispute this conclusion. In the homozygous animal carrying the weaver mutation but with a genetic background different from that of the mice studied by Rakic and Sidman, they find that Bergmann fibres appear almost normal. This was also found by Bignami and Dahl (*J. comp. Neurol.*, **155**, 219; 1974) who studied the development of Bergmann fibres in various neurological mutants using an immunofluorescence technique. Furthermore, the few granule cells that do migrate successfully undergo a similar course of degeneration as the postmitotic cells that fail to migrate.

Although the primary target of the genetic abnormality thus remains unsolved, its consequences on synaptogenesis have been analysed by Sotelo (1975) and Rakic and Sidman (*J. comp. Neurol.*, **152**, 103, 133; 1973). Because mutant cerebellum is agranular, the mossy fibre is deprived of its normal postsynaptic target, and apical dendrites of the Purkinje cell lack the afferent terminals which would occupy these synaptic sites. Probably because of the existence of unoccupied synaptic sites calling for innervation, there is an extensive remodelling of the cerebellar circuitry, as well as reshaping of the dendritic geometry. Instead of assuming the normal pattern of the dendritic tree with profuse tertiary branches, the Purkinje cell dendrites are oriented haphazardly and their thickened primary and secondary branches, without the usual spiny branchlets, continue to grow to the pial surface where they may turn inward. By contrast, the Purkinje cell dendrites in normal animals seem to recognise some kind of invisible barrier that excludes them from penetrating into the external granular layer. These aberrant, even inwardly oriented, dendrites are nevertheless entwined faithfully by climbing fibres, but their apical segments which are normally occupied by parallel fibres are frequently innervated by climbing fibres also. Failing to find their proper targets, mossy fibres extend their terminals and penetrate towards the superficial half of the cerebellum. The endings may then be encapsulated by dendrites of other types of neurone with which they normally do not form exclusive synaptic junctions.

These observations highlight some of the unsolved problems in neurobiology. The development of the nervous system involves a number of events

occurring in sequence. From the germinal layer, neurones migrate to their eventual destinations and extend dendritic and axonal arborisations characteristic of each cell type before functional connections are made between one neurone population and another. Since the cerebellar disarray in the weaver mouse results from the failure of granule cells to migrate, other neurones should be normal in appearance if their properties are determined solely by intrinsic epigenetic mechanisms. That the shape of Purkinje dendrites is drastically altered, and mossy and climbing axons extend their processes further than they normally do, suggests the importance of an interaction between a neurone and its milieu in the moulding of the nervous system. Moreover, the observation that synaptic contacts can be formed in the weaver cerebellum between pre- and post-synaptic elements which normally do not form junctions, shows that intrinsic specificity for afferent-efferent coupling can easily be overridden by an altered environment. Such a flexible strategy in the developmental programme is indeed one of the ways of minimising the effects of a previous mistake, genetic or epigenetic, in the subsequent formation of the nervous system. □

Thermodynamic errors by international commission

from D. R. Wilkie

ALAS, the gulf still seems wide between some branches of physiology and the base of physical chemistry on which biology must ultimately rest. Even so it is hard to understand how a distinguished international group comprising 11 physiologists (after consultation with 57 others) came to put forward recommendations that are simply at variance with the laws of thermodynamics. Even more remarkable, these recommendations have apparently remained unchallenged for the one and half years since they were first published, suggesting that confusion about this topic is indeed widespread.

The International Commission of the IUPS for Thermal Physiology has published detailed recommendations concerning the names and units to be employed by physiologists engaged in studying metabolism, thermal balance, and so on (Bligh and Johnson, *J. appl. Physiol.*, **35**, 941; 1973). This is a praiseworthy enterprise, and the Commission has made one or two radical recommendations, the most striking (page 941) being

that "In the BODY HEAT BALANCE EQUATION, the symbol M is now used to denote METABOLIC FREE ENERGY PRODUCTION; METABOLIC HEAT PRODUCTION is identified by the symbol H ". By "free energy production" is clearly meant the rate of change of free energy associated with the chemical processes of metabolism, taken with a negative sign. This new definition has much to commend it since it is the changes of free energy, rather than of energy or enthalpy, that determine the potentiality of chemical reactions to drive the processes essential to life—such as biosynthesis and thus growth, production of electrical or mechanical work, and active transport.

The proposal is somewhat idealistic since free energy changes are usually difficult to measure. There are abundant calorimetric determinations of the energy and enthalpy changes associated with the oxidation of foodstuffs, but I at least do not know of similar systematic determinations of free energy changes since the pioneering work of Burk (*Proc. R. Soc.*, **B104**, 153; 1929) who showed for the oxidation of carbohydrate that the free-energy change was almost equal to the enthalpy change. It cannot be assumed that the same near equality applies for the oxidation of other foodstuffs: and it certainly does not apply for other important metabolic reactions such as the hydrolysis of ATP. In any case, when one is concerned purely with questions of energy balance (as in most of thermal physiology), the free energy change is not directly relevant. Confusion over this point has led to the incorrect equation (recommendations page 943):

"BODY HEAT BALANCE EQUATION: A mathematical expression that describes the net rate at which a body generates and exchanges heat with its environment (First Law of Thermodynamics):

$$S = M \pm E - (\pm W) \pm R \pm C \pm K$$

[W] or [W m⁻²]

in which

S = rate of STORAGE OF BODY HEAT (+ for net gain by body)

M = METABOLIC FREE ENERGY PRODUCTION (always +)

E = EVAPORATIVE HEAT TRANSFER (− for net loss)

W = WORK (+ for POSITIVE WORK against external forces)

R = RADIANT HEAT EXCHANGE (+ for net gain)

C = CONVECTIVE HEAT TRANSFER (+ for net gain)

K = CONDUCTIVE HEAT TRANSFER (+ for net gain)."

This equation is incorrect. It is compatible with the First Law only if one substitutes for their definition of M :

" M = the total rate of change of enthalpy resulting from the chemical processes of metabolism, taken with a negative sign".

I have since confirmed in a helpful correspondence with Dr Bligh that the Commission was not fully aware of the fundamental distinction between 'free energy' and 'energy' or 'enthalpy'. Clearly it will take some time to frame modified recommendations that do take account of this difference: meanwhile the recommendation quoted from page 943 (and others derived from it on pages 949, 950, 951 and 958) should be interpreted with great caution.

Basically the relation between the chemical processes of metabolism and their physical manifestations, work produced (w) and heat produced (h), is perfectly simple, (Wilkie, *Prog. Biophys.*, **10**, 260; 1960). In modern terminology we have, under strictly isothermal conditions, over a defined time interval Δt :

$$h + w = \Delta \xi_1 (-\Delta H_{m1}) + \Delta \xi_2 (-\Delta H_{m2})$$

and so on

$$= \sum_0^j \Delta \xi_i (-\Delta H_{mi}) \quad (1)$$

where the subscripts 0– j designate the various chemical processes occurring; the $\Delta \xi_i$ represent the extents by which each reaction has advanced (measured most conveniently as additional mol of a named product that have appeared); and the ΔH_{mi} are the molar enthalpy changes of the various reactions measured calorimetrically and expressed in kJ per mol of the same named product formed.

Note that if the organism is in a steady state—and the experimental design in most metabolic studies aims to achieve this—the situation becomes very simple indeed since the $\Delta \xi_i$ of all the intermediary reactions are then zero. If only a single substrate (carbohydrate for example) is being oxidised the right hand side of equation (1) then becomes reduced to a single term (Wilkie, *J. Mechanochem. Cell Mot.*, **2**, 257; 1974). At first sight this may seem surprising since the intermediary reactions are still proceeding and contributing to the heat produced. That they are equivalent to a single global reaction is a consequence of Hess's Law.

Thermal physiologists are generally not interested in strictly isothermal conditions since they are concerned with what happens when the body temperature θ changes. They are also concerned with the various ways in which heat can be transferred from the body to the environment by evaporation (e), by radiation (r), by convection (c) and by conduction (k). Equation (1) can be readily adapted for this purpose. Rearranging into roughly the same form as that quoted from the recommendations:

$$C\Delta\theta = \sum_0^j \Delta \xi_i (-\Delta H_{mi}) - w - e - r - c - k \quad (2)$$

C is the thermal capacity of the body (SI unit $J^\circ C^{-1}$) so the left-hand side is the change of thermal energy, the "storage of body heat". All the terms in the above equation are quantities of energy (J): each can be converted to power (W) by dividing by Δt .

Note that when work is done on the organism rather than by it (so-called "negative work") w simply appears in Equations (1) and (2) with its sign changed.

Consequently there seems to me to be no need to introduce the distinction between "TOTAL HEAT PRODUCTION" and "METABOLIC HEAT PRODUCTION" presented in the recommendations; it merely confuses further a

situation that is already confused enough

One final point of criticism is that I think that the Commission showed poor judgement in endorsing the use of the phrase "energy metabolism" (pages 946 and 951). It would be far safer to restrict the word "metabolism" to the chemical events in living organisms so as to maintain a clear distinction between the chemical changes and the changes of physical energy that result from them. This is not merely a matter of semantics. Confusion by physiologists over this distinction has recently provoked the misguided suggestion (Banks and Pearce, *Lancet*, **i**, 811; 1975) that energy changes are irrelevant in considering metabolic processes. $\square$

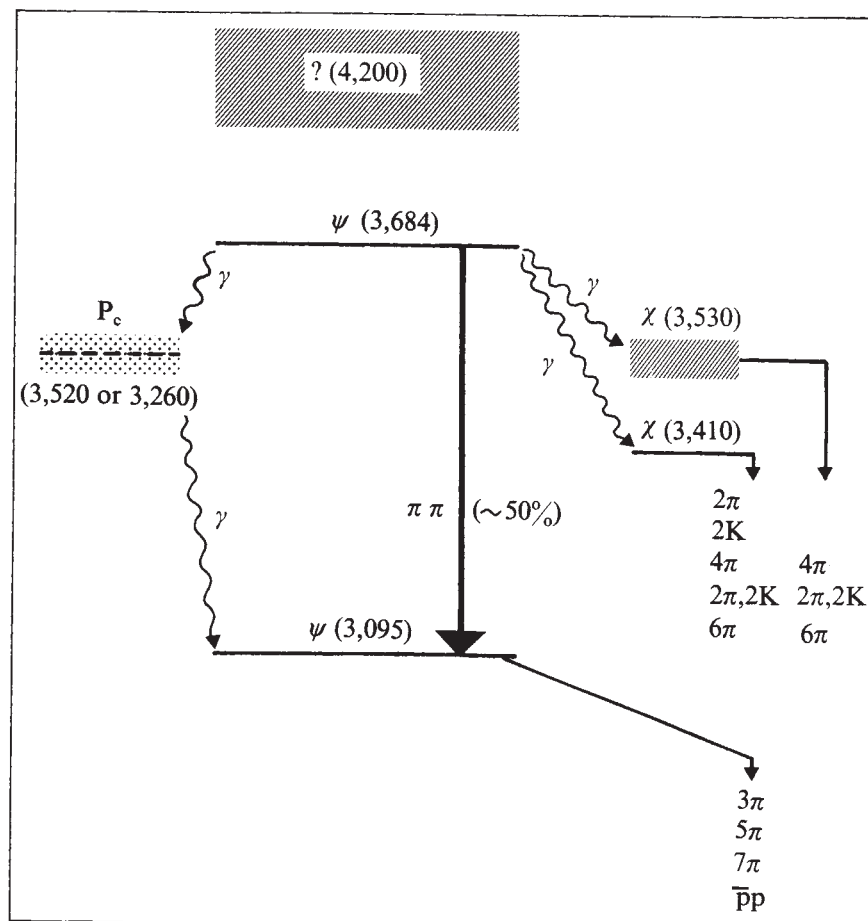
More new particles

from W. Toner

Two remarkably long-lived new particles were discovered last November: the ψ (mass 3,095 MeV), alias J, and the ψ (3,684). At least two new states related to the ψ s, have now been observed in detailed studies of the decays of the ψ

(3,684), and it begins to be possible to sketch a tentative level scheme, as shown in the figure. A preliminary report of these studies was given in *News and Views* for August 21.

The evidence for the state named P_c comes from a group working at the electron-positron storage ring DORIS, in Hamburg and is discussed in a paper by Braunschweig *et al.* (*Phys. Lett. B*,



Tentative level scheme (July/August 1975). Only a small fraction of the decays have been identified, and only a few of those are shown. The 3,095, 3,684 and 4,200 are formed directly in electron-positron annihilation. P_c may (or may not) be the same as $\chi(3,530)$.

57, 407; 1975). They have shown that a few per cent of ψ (3,684) decays lead to the formation of the ψ (3,095) by the emission of two γ rays. They also have data which strongly suggest that the decay proceeds through an intermediate state (or cluster of states) which they have called P_c . At present they do not know whether the more energetic of the two γ rays is emitted first or second, so that their mass determination is ambiguous.

The states named χ in the figure were discussed at the recent Lepton-Photon Symposium at Stanford by Feldman *et al.*, the Stanford-Berkeley collaboration working at the SPEAR ring in Stanford, California. This group discovered the ψ (3,095) (simultaneously with Ting, at Brookhaven, who called it the J) and the ψ (3,684). They observe that a few tenths of a per cent of ψ (3,684) decays proceed by the emission of a γ ray and two, four or six strongly interacting charged π and K mesons. In the decay ψ (3,684) $\rightarrow \pi^+ \pi^- \pi^+ \pi^- \gamma$, the four pions form one or other of two very distinct states χ (3,530) and χ (3,410) whereas in ψ (3,684) $\rightarrow \pi^+ \pi^- \gamma$, the two pions form only the lower state, which is narrow (that is, long-lived). Both states appear to be formed in the 6π and 2π 2K channels. The upper state is broad, and may be a group of states. It may (or may not) be the same as P_c .

The discovery of these states ends a period of uncertainty about the general nature of the mechanism which gives the ψ s their remarkably long lives. Any explanation invoking a new quantum number whether 'charm' or 'colour' or something else inevitably required there to be a complex set of states, some of which could be reached by the emission of a γ ray from a ψ . Indeed, most models predicted much greater radiative decay rates than are observed, and inspired the earlier fruitless searches at lower sensitivity which gave rise to the uncertainty. Now that they have been seen, 'charm' is once again a hot favourite, and a multitude of detailed models is being developed in which the 'obvious' decay modes are suppressed by one subtlety or another. In these models, the ψ s, χ s and P_c though made of a charm-anticharm pair of quarks have charm = 0. Overtly charmed particles with charm = ± 1 would have to be produced in pairs. Again, fruitless searches have been carried out for theoretically obvious decay channels, and the models are now being adjusted accordingly. Colour enthusiasts have not yet given up, and of course, it could be something else altogether.

The experimenters need not involve themselves in the theoretical controversy and are being careful about the precise wording of their interpretations. For example, the broad? (4,200) state shown in the sketch is not claimed as a particle

by the Stanford-Berkeley group. They describe it as an enhancement which could be one or more new particles or, possibly, an effect connected with the threshold for some other new phenomenon. They are assured that their data—and they have a great deal more in the course of analysis—will have the last word. □

The peptide hormones: molecular and cellular aspects

from D. G. Smyth

Biosynthesis and release of secretory hormones formed the subject of a Symposium arranged by the Ciba Foundation in honour of Sir Frank Young (London, June 30–July 3). The proceedings are to be published in full by Associated Scientific Publishers, Amsterdam.

It is beginning to be accepted that most if not all peptide hormones are biosynthesised as fragments of larger molecules. The search is now on for the ultimate precursors, synthesised on the ribosome and translated from messenger RNA. J. F. Habener (Massachusetts General Hospital) described the primary biosynthetic form of parathyroid hormone, the 'preproparathyroid hormone', which contains a peptide of 31 amino acids attached to the N-terminus of the 84-residue hormone. The major part of the extension, comprising the first 25 amino acids, is excised shortly after biosynthesis and before transference of the 90-residue prohormone to the secretory granule. The existence of an analogous precursor to insulin, a preproinsulin, was strongly supported by the experiments of M. A. Permutt (Washington University) who isolated RNA from catfish islets and observed its ability to stimulate protein synthesis in a wheat germ cell-free system: among the proteins formed was a major component with a molecular weight of 11,000, larger than catfish proinsulin. The *in vitro* synthesised product shared antigenic determinants with catfish insulin and appeared to be identical with a rapidly labelled protein synthesised by the islet tissue. From mammalian pancreas the isolation of mRNA has proved more difficult because the islets comprise only 1% of the tissue and ribonuclease is highly active. D. F. Steiner (University of Chicago), working with a microsomal fraction from rat islets, concluded that the signal peptide must be detached from the preprohormone on the ribosome before the proinsulin

chain is completed. He suggested there may be two distinct enzyme-catalysed steps in the export of secretory hormones, the pre-processing stage which does not involve a trypsin-like enzyme and the pro-processing stage which does. The conversion of some prohormones to hormones, however, may involve enzymes with specificities different from trypsin, and activation may take place not by hydrolysis but by aminolysis of the peptide chain (D. G. Smyth, NIMR, Mill Hill). This transamidation reaction would account for the origin of the C-terminal amide that is a characteristic feature of many of the peptide hormones and both the known releasing factors. On this basis it would be predicted that LHRF, TRF, gastrin, oxytocin, vasopressin, secretin, α -MSH, and others, will be found to arise from prohormones or pro-releasing factors that extend from the C-terminal residues.

β -MSH

The fragments formed by enzymic activation of a prohormone are retained in the secretory particle of the gland. From pig pituitary, β -MSH and two novel peptides were isolated in equivalent amounts; the three peptides represented contiguous fragments of the 91-residue polypeptide termed β -LPH and appear to have been released by an enzymic mechanism similar to that involved in the activation of proinsulin (A. F. Bradbury, D. G. Smyth and C. R. Snell, NIMR). On this evidence, it was proposed that β -LPH is the prohormone of the lipolytic hormone β -MSH. Final proof of the relationship between β -MSH and β -LPH will be obtained by isolation of the prohormone from the secretory granule and study of its conversion by granular enzymes. It is of interest that β -LPH and its 58-residue fragment γ -LPH, which are only weakly active as lipolytic agents, are present in substantial quantity in pig pituitary and this raises the possibility that the enzymic release of the hormone from these precursors may be under physiological control. Lesley Rees (St Bartholomew's Hospital) reported that β -MSH could not be detected by immunoassay of human pituitary extracts and maintained that in the human the physiological significance of this hormone is in question.

ACTH

Precursors to several hormones have been indicated by immunoassay of glandular extracts or plasma (Rosallyn Yalow, Veterans Administration Hospital, Bronx). The high sensitivity of the method allows analyses to be performed on serum from a single individual but inevitably the conclusions drawn are limited to assessments of

molecular size and convertibility of the products. A 'BIG' ACTH was found in tissue extracts of carcinoma primary to or metastatic from the lung. Excluded from Sephadex G50 and with low corticotrophic activity, 'BIG' ACTH was converted by controlled tryptic digestion to a product with high activity, apparently identical in size and charge to (1-39) ACTH. Curiously, an intermediate size ACTH seems to be present in rabbit, rat and mouse whereas the usual 39-residue peptide predominates in other species. Yalow suggested that the hormonal form of ACTH may be a factor in regulating the cortisol:cortisone ratio in mammalian corticoid secretion. Working with pig pituitary, Bradbury *et al.* (NIMR) isolated and sequenced a new 38-residue peptide which was present in large amounts; they considered that it may be part or all of the N-terminal region of a prohormone to ACTH.

Gastrin

A 'BIG' gastrin, predicted by S. A. Berson and Rosalyn Yalow, has now been isolated from antral mucosa or

Zollinger-Ellison tumour tissue (R. A. Gregory, Liverpool University) and its sequence determined. In this precursor, a heptadecapeptide extends from the N-terminus of the 17-residue hormone and, as with the prohormones of insulin, glucagon, PTH and β -MSH, it is clear that activation is by enzymic cleavage at the C-terminal side of paired basic residues. The list of gastrins and gastrin precursors has grown to include component 1 (Cl), 'big big' gastrin (BBG), 'big' gastrin (BG, 34 residues), little gastrin (LG, 17 residues) and minigastrin (G, 13 residues); all these are duplicated by corresponding forms with sulphated tyrosine. In addition, it is known that the C-terminal pentapeptide retains full activity.

Hormone activity regulation

P. J. Randle (University of Bristol) suggested that the regulation of hormone activity may take place not by control of the rate of enzymic attack on a prohormone but by alteration of specificity. Covalent modification of certain enzymes by phosphorylation,

which is sensitive to calcium, could lead to the formation of prohormone fragments that differ in size, potency, and possibly in duration of action. In general, the conversion of prohormones to hormones is being found to take place by way of numerous intermediates, some stable some transient. Only when these have been isolated in homogeneous form and subjected to biological scrutiny will the ground be cleared for understanding the physiological role of each component.

In the closing presentation, J. R. Tata (NIMR) called attention to two classes of response initiated by peptide hormones. Rapid responses involve change in permeability in the membranes of target cells, directly affecting the levels of intracellular intermediates; slower effects take place through stimulation of RNA and protein synthesis. It seems likely that different classes of hormone receptor are concerned. Tata proposed that the ultimate expression of growth and maturation results from the cooperative interaction of the two types of response. □

DESPITE the availability of undisturbed sites, the arctic tundra has, until recently, been one of the most understudied of the world's terrestrial biomes. Even now there are many leading questions concerning general ecosystem function and balance within this biome which remain unanswered. Studies of primary production by arctic ecosystems, both in the field and in the laboratory, have been receiving some attention recently, for example by Larry Tieszen at Barrow, Alaska (*Arctic and Alpine Res.*, 4, 307; 1972, and 5, 239; 1973) and results have now been published of a carbon dioxide flux study which has been undertaken at the same locality by Coyne and Kelley (*J. appl. Ecol.*, 12, 587; 1975).

The study of carbon dioxide flux between soil, vegetation and the atmosphere has certain useful attributes as a method of approaching gross ecosystem energetics. In particular it avoids the problems of extrapolating from small samples studied in detail, either by cropping, or by CO_2 exchange studies in enclosed conditions, or by $^{14}\text{CO}_2$ incorporation studies. The analysis of data obtained is difficult, however, because of both the meteorological and the biological variables involved. In effect, a flux of CO_2 from ground to atmosphere means that respiration of producers, consumers and decomposers exceeds photosynthetic consumption at that point

in time. The reverse is true if CO_2 flux is from atmosphere to vegetation.

Coyne and Kelley worked through an arctic growing season (mid-June until the end of August) and regularly took air samples at five heights

Carbon dioxide flux in arctic ecosystems

from Peter D. Moore

up to 16 m, which they analysed for CO_2 concentration using infrared gas analysis. They also monitored various micrometeorological variables, such as wind speed, light and air temperature. For much of this season, the ecosystem received light for a full 24 h (diurnal variation, about 50 W m^{-2} at night to 250 W m^{-2} at mid-day). Diurnal air temperatures varied from 4 to 24°C . CO_2 flux also varied diurnally; on July 21, midnight values were about $-0.2 \text{ g m}^{-2} \text{ h}^{-1}$ (that is, flux from ground to atmosphere), and at mid-day, about $+0.6 \text{ g m}^{-2} \text{ h}^{-1}$. For about 17 h of the day in late July there was a positive flux of CO_2 from the atmosphere to the vegetation. For the remaining 7 h, photosynthesis failed to consume all of the CO_2 produced by ecosystem respiration. In late June and early August, the negative flux periods

were of 12-14 h duration per day. In these early and late season times, there was a daily net flux of about $-1 \text{ g CO}_2 \text{ m}^{-2}$. In the height of the season daily net flux was $+8 \text{ g CO}_2 \text{ m}^{-2}$.

Over the entire season (61 d), it was calculated that $626 \text{ g CO}_2 \text{ m}^{-2}$ was generated by ecosystem respiration. This would largely be due to the primary producers and the decomposers, the latter also responding to raised temperatures by increased activity. During the season $146 \text{ g CO}_2 \text{ m}^{-2}$ (net) was consumed by primary producers in photosynthesis; thus gross production would amount to $772 \text{ g CO}_2 \text{ m}^{-2}$. If one applies the crude conversion factor from CO_2 incorporated to dry weight accumulated of 0.614, then this represents 474 g m^{-2} dry matter (gross) in a season. The net production figures compare reasonably with Tieszen's values obtained by more conventional methods, which lends credibility to the data derived from these flux studies.

This new approach has thus provided further information concerning the consumption and generation of CO_2 in the tundra ecosystem. If the ecosystem is in a stable, equilibrium state, then one must assume that the net seasonal gain of $146 \text{ g CO}_2 \text{ m}^{-2}$ is either being returned to the atmosphere by off-season decomposition or is accumulating as litter or peat within the system.

Interstellar dustmen's convention

from Ant. Whitworth

A workshop on Interstellar Grains was organised by University College Cardiff at Gregynog Hall on July 11-13.

DUST gets into everything, or so they always say, and certainly it seems the cosmos is no exception. In astrophysics, dust grains (sub-micron sized particles constituted of compounds of Si, C, N, O, Fe, Mg) are continually invoked, and invested with new capabilities, to explain puzzling observations. Yet the recent increase in constraints on dust models has not resulted in a more definite picture of dust grains, but rather in the introduction of more free parameters (such as multi-component models and localised variations). Nevertheless, a pattern emerges, and with it the realisation that grains have many very fundamental roles in the cosmos.

At the recent workshop H. C. van de Hulst (Sterrewacht, Leiden) stressed that the interstellar medium is a very hostile environment for grains; and J. M. Greenberg (Sterrewacht, Leiden) pointed out the need to consider how intense irradiation may modify grain properties. J. P. Bibring (Laboratoire René Bernas, Orsay) has investigated grains in lunar and meteoritic samples, and concluded that irradiation of grains in circumstellar environments—say by stellar winds, energetic photons, or cosmic rays during their formation—may significantly round the grain and amorphise its internal structure. D. R. Huffman (University of Arizona, Tucson) pointed out that this bears on the identification of the $10\text{ }\mu\text{m}$ feature in infrared spectra which requires amorphous silicates; whereas the $2,200\text{ }\text{\AA}$ ultraviolet feature requires well-ordered graphite. S. Ramadurai (Institute of Astronomy, Cambridge) argued that carbonaceous chondrites contain interstellar graphite grains from the protosolar nebula, as evidenced by their anomalously high boron content, which is attributed to cosmic ray bombardment of graphitic carbon.

But a cogent case was made by N. C. Wickramasinghe (University College, Cardiff) for considering grain materials with a volatility intermediate between the very volatile 'dirty ices' (hydrides of C, N and O), and the more refractory silicates and graphite. He cited organic polymers, and in particular suggested that conditions in dark clouds may favour condensation of the widespread formaldehyde mole-

cule into elongated grains of polyoxymethylene (POM). There remains outstanding the problem of producing sufficient formaldehyde, but there is already good evidence in support of the general thesis. D. A. Mendis (University of California, La Jolla) reported on the physical parameters for the grains producing 10 and $18\text{ }\mu\text{m}$ features in comets, and indicating an evaporation temperature $\sim 500\text{ K}$: this rules out silicates, but is very compatible with many polymers, including POM. B. Thomas and co-workers (University of Wales Institute of Science and Technology, Cardiff) have measured strong absorption bands around 10 and $18\text{ }\mu\text{m}$ in thin films of POM.

M. J. Barlow (Sussex University) proposed a resolution of the problem of supplying sufficient refractory grains to the interstellar medium: refractory grains condense in almost every stellar mass loss situation, and are probably only destroyed by direct involvement in star formation after a mean lifetime $\geq 10^9$ yr. D. P. Gilra (University College, Cardiff) deduced the existence of a temperature minimum in the atmospheres of late N-type carbon stars where SiC_2 grains are condensing. C. D. Andriesse (Sterrewacht, Roden) presented infrared observations of η Car as evidence of dust suddenly condensing in the mass outflow at a radius ~ 0.03 pc. H. Okuda (Kyoto University) interpreted polarisation data on VY Can Maj in terms of a circumstellar dust disk: is this disk protoplanetary? J. Silk (University of California, Berkeley) prescribed a scenario for fragmenting and reconstituting dust in cocoons around newly formed massive stars.

W. W. Duley (York University, Ontario) offered new hope for identifying the diffuse bands: he attributed narrow lines to impurities in the bulk grain material, and the adjacent broader features to the same impurities in grains which are so small that their lattice constant is altered. D. P. Gilra (University College, Cardiff) calculated infrared absorption cross sections for small ellipsoidal particles, taking account of surface modes, and stressed the great importance of grain shape. A. P. Whitworth (University College, Cardiff) argued that in neutral clouds dielectric grains are charged stochastically on a time scale $\sim 10^3$ s, typically having $0, \pm 1$ electron charges. Consequently, grains are effectively untied from the magnetic field, can move through the gas under gravity and/or radiation pressure, and can be aligned in the required sense. P. A. Aannestad (University of Arizona, Tucson) suggested that stochastic grain charging may also enable grains to deplete C^+ , N^0 and O^0 equally, as inferred from observation. But D. A. Williams (Uni-

versity of Manchester Institute of Science and Technology) concluded that the depletion of CNO may anyway be negligible.

A number of interesting roles have been suggested for grains in cosmology. A. J. R. Prentice (University of Oxford) argued that in the rotating protosolar cloud, grains fall to the centre creating a core sufficiently massive to stabilise the rest of the cloud, and establishing composition gradients which will explain the solar neutrino deficiency. I. P. Williams (Queen Mary College, London) has shown the basic part which grains may play in forming the planets and determining their different compositions. M. Rowan-Robinson (Queen Mary College, London) poses the question: is dust always associated with violent events in galactic nuclei? One might also ask whether such events are not a major source of the dust in the cosmos. □

Galaxy formation

from Janet E. Jones

A workshop meeting on "The Formation and Evolution of Galaxies" was held at the Institute of Astronomy, Cambridge, on August 4-8, and supported by the Gravity Research Foundation.

LEAVING aside the still controversial topic of the origin of pre-recombination density inhomogeneities, the emphasis at the workshop was focused on later epochs, with theories for early galactic evolution and observational "clues" on the topic being well discussed. The workshop opened with a number of papers on the covariance and multiplicity functions, the purpose being to use the presently observed spatial distribution and luminosity functions of galaxies as indicators of the primordial density fluctuation spectrum.

It was pointed out that non-linearities would tend to increase the ultimate slope of the covariance function, thus making it more difficult for an initially flat, $n=0$ (Poisson), density fluctuation spectrum to account for the observed covariance function: an $n=-1$ initial spectrum seems to give a better fit (J. R. Gott and M. Rees, Institute of Astronomy, Cambridge). Moreover, the slope of the covariance function seems to depend on galaxy type, being steeper for ellipticals than for spirals (M. J. Geller, Harvard University). This could suggest some difference in the primordial density fluctuation spectrum leading to the two types, although later attempts to model galactic evolution did not incorporate this property. In-

deed, a notable non-feature of the workshop was the absence of any attempt to propose a genuine bifurcation scheme for elliptical versus disk-like galaxies. P. Schechter (Institute for Advanced Study, Princeton), W. Press (Princeton University), Gott and Geller all discussed aspects of the multiplicity and luminosity functions for galaxies. The multiplicity function for the Gott-Turner groups of galaxies seems to be consistent with an initial Poisson density fluctuation spectrum (Schechter), although, as with the covariance function, an initial $n = -1$ spectrum gives a slightly better fit (Gott).

B. Jones (IOA, Cambridge) reviewed turbulence theory for the origin of primordial density perturbations, and discussed the effect of the recombination process on the covariance function derived from both the cosmic turbulence theory and the adiabatic density perturbation theory. This, and a later more specialised discussion by S. Bonometto (University of Padua), emphasised some of the difficulties inherent in both these theories for the origin of primordial density inhomogeneities.

The discussion then moved on to the topic of galactic evolution. It was argued that galaxies evidently originate from a single protogalactic cloud, since the globular clusters display a range of properties that vary systematically through the Galaxy, and from one galaxy to another (S. van den Bergh, University of Toronto). But this "monolithic" unit may have increased in size as a result of infalling material, as suggested by measurements of stellar metallicities in the solar neighbourhood (D. Lynden-Bell, IOA, Cambridge). Direct calculation of the collapse of a turbulent protogalactic cloud led to fragmentation into $10^{6-7} M_{\odot}$ clouds, with the formation of a massive central star in each cloud fragment, inhibiting further star formation (B. Jones, and S. Weber, University of California, Berkeley). It is not clear how further evolution would proceed from this point. At any rate, the two theories of galactic evolution presented at the workshop each included an assumed star formation rate without regard to the detailed physical processes involved. The first model by Gott and T. X. Thuan (CalTech) took as its starting point a rotating protogalactic cloud, and assumed that star formation occurred over some characteristic timescale, T_{st} . If this were short compared with the collapse timescale of the galaxy, T_{tr} , then the result was an elliptical system, and if long ($T_{st} > T_{tr}$) it formed a disk system. The second model (R. Larson, Yale University) began with a turbulent protogalactic gas cloud, in which star formation took

place at an assigned rate during collapse. If the cloud had low angular momentum an elliptical system was formed, and if high angular momentum, a disk system formed, though some additional adjustment of other parameters was required in the latter case.

Neither model is perfect. Evidently, elliptical systems are easier to make than spiral ones, though even here there were some notable discrepancies.

Gott's model being dissipationless produced a majority of EO systems though it had the nice property that the maximum flatness produced was E7 (Gott and Thuan). Observations suggest however that E3 is the most common type. Conversely Larson's model tended to produce too much flattening. In each case, flattening was said to have derived from rotation.

But both models gave the observed luminosity profile for elliptical galaxies, assuming a constant mass to light ratio. Various studies suggest, however, that the mass to light ratio may vary; it is early days yet for the galaxy builders.

The situation as regards the formation of spiral galaxies is even less satisfactory. There seems to be some difficulty in producing a clear division of type between ellipticals and spirals when starting with the same initial conditions and continuously varying parameters. Van den Bergh pointed out that SOs were not truly intermediate in type between the two classes, their ellipticity and luminosity profiles both being characteristic of spirals. He emphasised the value of the bulge/disk ratio as an indicator of spiral type and it was even suggested (Ostriker and Faber) that all galaxies could be classified by means of their elliptical component, going from large ellipticals, through spirals, to dwarf elliptical systems. But the mechanism by which the disk is produced is not clear.

Faber suggested that the bulge/disk ratio may be a factor in the removal of gas, arguing for the presence of a galactic wind in spheroidal systems; however, van den Bergh pointed out that this could not be the only factor involved in gas loss, since ellipticals and SOs are predominantly found in clusters. He therefore supported the idea that gas is stripped from galaxies by the ram pressure of an intergalactic gas. This process was proposed by G. R. Gisler (IOA, Cambridge) to explain the very low gas content found in most elliptical systems, where population synthesis studies indicate that more gas is being lost by stars than is evidently consumed by new star formation (B. Tinsley, Yale University, and W. O'Connell, University of Virginia). This assumes a normal stellar mass function, since there is no obvious way to manufacture only low mass stars.

But since the ram pressure effect could not easily account for the variations in gas content in spiral systems it seems likely that more than one process is operating to influence the gas content of galaxies in general.

The last problem to be considered was the possible detection of galaxies in the process of formation. An argument based on free-fall times suggested that galaxies would have formed between a redshift of 50 and 5, and perhaps as recently as $z=3$ (Larson). An elegant study by D. Meier (University of Texas, Austin) based on the models of Larson and Tinsley, predicted a possible spectrum for a newly formed galaxy, and indicated that its nucleus could be visible as a QSO. He proposed two candidate objects worthy of further investigation in this field: OH471 and 4C05.34. The principal argument against Meier's work is that it neglects the effect of dust. This is a significant omission, since if most of the radiation were absorbed by dust and re-emitted in the infrared, it may be difficult or impossible to detect (M. Rowan-Robinson, Queen Mary College, London). But what is particularly unfortunate for those engaged in the task of galaxy making is that there are apparently no reasonable candidates for galaxies in the process of formation now (W. L. W. Sargent, CalTech). Presumably, this implies that either there are no more suitable protogalactic clouds around, or that conditions are no longer right for turning them into galaxies, or both. In the words of Sidney van den Bergh: "Galaxies are like people: they depend on both genetics and environment". This, indeed, was the theme of the workshop. □



A hundred years ago

A UNIVERSITY is to be founded at Tomsk, one of the chief towns of Siberia. The new establishment will have only two faculties, one of Law and the other of Medicine. The want of doctors in Siberia may be inferred from the fact that there are only fifty-five of them in a country which is as large as the whole of Europe, and whose population amounts to more than 6,000,000 inhabitants. The Russian Minister of Finance has granted a credit of 40,000*l.* on the revenue of the State for the new establishment, which will raise the number of Russian Universities to eight.

from *Nature*, 12, 446; September 16, 1875.

review article

Unified gauge theories of elementary particles

E. Leader & P. G. Williams*

Recent theories which unite weak and electromagnetic interactions provide new hope for a unified theory of fields and their interactions. Experimental developments such as the discovery of neutral currents and the new psi particles lend encouraging support for some of the ideas embodied in these theories.

One of the principal goals of 20th century physics has been to obtain a unified understanding of all the basic interactions observed in nature. Einstein spent his later years attempting to unify electromagnetic and gravitational interactions. His failure, and that of some of his contemporaries, was one of the reasons why the pursuit of such grand theoretical syntheses lost its popularity. Instead the main effort of fundamental physics has been concentrated on less ambitious, more limited goals, studying each interaction more or less in isolation.

In the past three years unification has once more come into vogue through some quite remarkable theoretical and experimental developments in high energy physics. In this article we shall attempt to outline the ideas behind these developments.

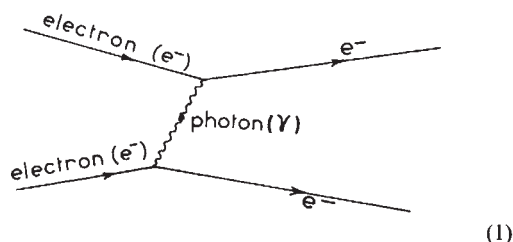
The past 40 years have led to the elucidation of four distinct types of forces or interactions acting between elementary particles: the strong, the electromagnetic, the weak and the gravitational forces, with relative strengths in the ratios $1 : 1/137 : 10^{-13} : 10^{-39}$. Whereas gravity acts upon all particles the other forces seem to be more selective. Table 1 lists a few of the known elementary particles and the forces they experience.

When hadrons interact it is difficult to study their weak and electromagnetic properties since their strong interactions are predominant. We cannot switch off the strong interactions; but nature has provided its own probe—the leptons—which seem to have no strong interactions. For the moment we shall confine our attention to the leptons, returning to hadron physics again later.

Electromagnetic interaction of leptons

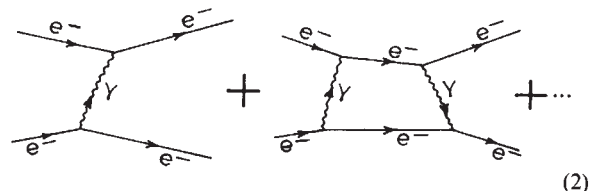
A typical leptonic electromagnetic process is the scattering of one electron by another: $e^- + e^- \rightarrow e^- + e^-$. Classically the two negatively charged electrons repel each other and are deflected from their original paths.

In quantum field theory the interaction is pictured as arising from the exchange of an electromagnetic wave (that is a photon) between the two electrons as in (1).

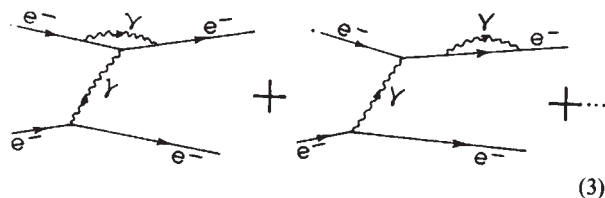


But this is not the only mechanism contributing to the scattering: two or more, in fact any number of photons can be

exchanged. The physical process is described by the sum total of all such mechanisms (2).



Each of these “Feynman” diagrams corresponds to a precise mathematical expression. Each photon exchanged represents one interaction with the electromagnetic forces. Fortunately these forces are so weak that the more photons there are in a diagram the smaller its contribution. The force produced by the exchange of a single photon depends on the strength of the “coupling” between the photon and the electron. This is equal to the electric charge, e , of the electron. In Fig. 1 the force is proportional to $e^2 = 1/137$. Thus the scattering process is described by a series of successively decreasing terms proportional to e^2 , e^4 , e^6 , and so on. Unfortunately some diagrams give rise to mathematical expressions which when evaluated are infinitely large. An example occurs at order e^4 where each of the three diagrams in (3) is infinite—but miraculously their sum is finite! Crucial to this cancellation of infinities is



the “universality” of the coupling of the photon to electrically charged matter: the fact that the force is always proportional to the electric charge, and is always the same; the electric charge is “conserved”. Quantum electrodynamics is riddled with these infinities, but they always cancel. A field theory with this remarkable property is said to be “renormalisable”.

It is sometimes said that the finiteness of the theory depends on the fact that the photon has zero mass; but this is not the case. One can imagine a theory of electrodynamics with massive photons and this is also renormalisable. That the mass of the photon is zero is an experimental fact intimately connected with the inverse square law, and known to great accuracy. A consequence of the zero mass of the photon is that electromagnetic waves are transverse, having only two possible polarisation states, both perpendicular to the direction of propagation.

* Address: Westfield College, University of London, London, UK.

Table 1 Properties and interactions of some elementary particles. Superscripts denote the electric charge in units of the electronic charge e : thus μ^+ has charge $+e$; μ^- charge $-e$

Generic name	Interactions	Particles	Antiparticles
Hadrons	Strong Electromagnetic and Weak	Proton p	Antiproton $\bar{p}$
		Neutron n	Antineutron $\bar{n}$
		Pions π^+ π^0	π^- π^0
Strange particles		Kaons K^+ K^0	K^- $\bar{K}^0$
		Lambda Λ^0	Antilambda $\bar{\Lambda}^0$
Leptons	Weak and electromagnetic	Electron e^-	Positron e^+
		Muon μ^-	Antimuon μ^+
	Weak	Electron Neutrino ν_e	Electron Antineutrino $\bar{\nu}_e$
		Muon Neutrino ν_μ	Muon Antineutrino $\bar{\nu}_\mu$

A massive "photon" could also have a longitudinal polarisation and therefore a total of three polarisation states. These states of longitudinal polarisation are additional sources of infinities when the "photon" has a mass, but they cancel as a result of charge conservation. That is why the electrodynamics of massive "photons" is also renormalisable.

To summarise: the quantum electrodynamics of electrons and photons is a complete theory in the sense that all physical processes involving only electrons and photons can be calculated to arbitrary accuracy by perturbation theory, and the results are finite. The experimental record of quantum electrodynamics is impeccable: there are no known deviations from the theory and some predictions agree with experiments to within parts per million.

We have remarked that the cancellation of infinities in quantum electrodynamics is miraculous. In the next section we shall examine the mechanism behind this miracle.

Gauge invariance

To every conservation law in physics there corresponds a symmetry of the dynamical laws. For example, energy and momentum conservation are consequences of the symmetry of physical laws under translations in time and space: angular momentum conservation is a result of symmetry under rotations.

Charge conservation, the root cause of the renormalisability of quantum electrodynamics, is connected with the fact that the electromagnetic potentials are not unique, but may be altered in a particular way at different points in space and time without any change in the form of the laws of electrodynamics. In a quantum field theory these changes in the electromagnetic potentials are always accompanied by a change in the fields that represent the particles; the particles react to the effect of the change in the electromagnetic potentials, but in just such a way as to leave the physics unaltered. This symmetry is termed gauge invariance. To emphasise that each point in space-time has its own different gauge transformation we use the nomenclature "local" gauge invariance.

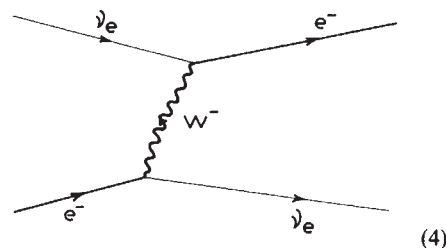
Not only does local gauge invariance lead to charge conservation, but it also forces the photon mass to be zero. But as we mentioned before massive photons also give a renormalisable electrodynamics, so that local gauge invariance is a larger symmetry than needed for charge conservation and for renormalisability. It turns out that all we require for charge conservation is a "global" gauge invariance where the gauge transformations are the same at all space-time points. The photon may have a mass without violating this symmetry.

This approach to charge conservation may seem rather formal, but it lends itself to generalisations that will lead to a theory for the weak interactions.

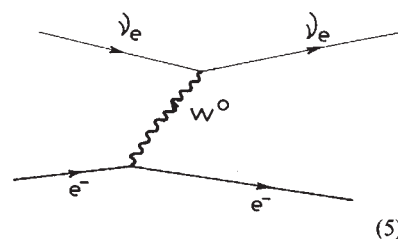
Weak interactions

At first sight it might seem that weak interactions have no features in common with electromagnetic processes. The β decay of the neutron into a proton, electron and antineutrino ($n \rightarrow p + e^- + \bar{\nu}_e$), the decay of muons $\mu^- \rightarrow e^- + \bar{\nu}_e + \nu_\mu$, are very different from typical electromagnetic interactions. Besides being far weaker, they violate parity conservation (that is left-right symmetry) and involve very short range forces; electromagnetic forces conserve parity and are long range as a result of the zero mass of photons. Yet Fermi, who first wrote down a theory of weak interactions in a remarkable paper in 1934, based his ideas on a close analogy with electrodynamics.

Let us imagine doing an experiment analogous to electron-electron scattering considered earlier, but involving only weak interactions. A good example involving only leptons is the elastic scattering of neutrinos off electrons, $\nu_e + e^- \rightarrow \nu_e + e^-$. By analogy with electrodynamics we shall assume that the weak force responsible for this scattering process is caused by the exchange of some "weak photon" called the W meson or W boson, as shown in (4). Note from the figure that since the neutrino has no electric charge, the W boson must have the same charge as the electron. As will be discussed later, until



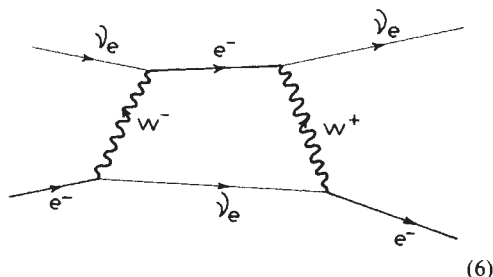
recently all experiments suggested that only charged W boson exchange occurred. If there were a neutral W boson, we could have also drawn a second diagram (5).



The first diagram (4) is called a "charged current" interaction; the second (5) a "neutral current" interaction. The latter bears the closest relation to electromagnetism because the photon also has no charge.

Now it can be shown that the range of the force generated by the exchange of a particle is inversely proportional to its mass. Experiment shows that the weak interactions are so short ranged that the W boson mass must be greater than that of any known particle. (Fermi's original theory corresponds to an infinite mass W.) Our failure so far to observe any W bosons could well be because our accelerators are not energetic enough to create them in the laboratory. Their discovery would constitute by far the most decisive evidence in favour of the picture we have painted of weak interactions.

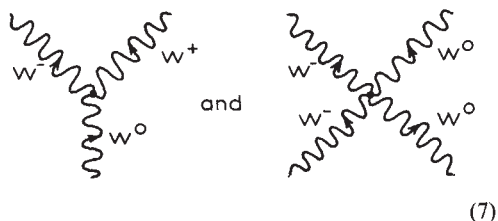
The theory outlined above—both in Fermi's original form and with a sufficiently large W mass—has been most successful in correlating the various phenomena of weak interactions. But it only works in the lowest order of perturbation theory. In our example of neutrino-electron scattering, the next most complicated term to be added to (4) is shown in (6) and is infinite.



Unlike quantum electrodynamics there is no way of cancelling the infinities of the theory—it is non-renormalisable. The trouble lies in a failure to pursue the analogue with electrodynamics closely enough: the theory contains a "weak photon" (the W boson) but there is no "weak charge" conservation, and the experience of quantum electrodynamics suggests that this is the ingredient needed to obtain a renormalisable theory. This problem was solved by Yang and Mills in 1954.

Yang-Mills gauge theories

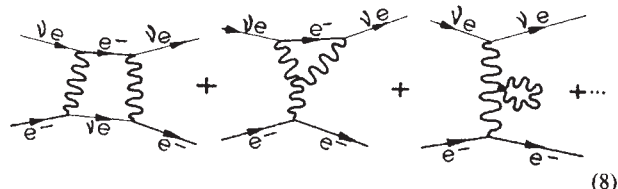
The difficulty of constructing a gauge invariant theory of the W bosons is that unlike the photon they must have electric charge. The photon, which only interacts directly with charged particles will not interact directly with itself. But Yang and Mills showed that because W bosons are charged they must interact with themselves and to achieve a gauge invariant theory one must also have a neutral W^0 in addition to W^+ and W^- . The possible diagrams are much more complicated now because three or four Ws can interact with each other as shown in (7).



These Yang-Mills theories are a natural extension of electrodynamics, because they possess the full local or space-time dependent gauge invariance only when the W bosons are massless, but the introduction of masses still preserves the conservation of a "charge". This is, of course, not the electric charge but something we shall wish to identify as the weak charge alluded to in the previous section. We have already mentioned that electrodynamics with massive photons is renormalisable because charge conservation is sufficient to guarantee this without need for the full local gauge invariance.

Weak interactions are short range and therefore require massive W bosons. The analogy with electrodynamics with massive photons suggests that a massive Yang-Mills theory will be renormalisable and, therefore, could provide the basis for a theory of weak interactions.

At first it might seem that massive Yang-Mills theories are indeed renormalisable. Thus, in the example of elastic neutrino-electron scattering the introduction of a self-interacting W boson leads to the addition of further diagrams to the infinite two W boson exchange diagram (8).



All these diagrams are individually infinite, but their sum is finite—the infinities cancel out exactly. Unfortunately it was later shown that such cancellations do not always occur and therefore massive Yang-Mills theories are not, in fact, renormalisable. It turns out that the full local gauge invariance is a necessary requirement and this means that the W bosons cannot be given a mass, although experiment requires it.

The inescapable conclusion seemed to be that gauge theories, except in the simplest case, electrodynamics, do not apply to the real world. In 1971, however, a Dutch graduate student, 't Hooft, opened the flood-gates by giving convincing arguments for the renormalisability of a rather special type of Yang-Mills gauge theory. Such theories circumvent the difficulties described in this section by an ingenious and elegant device.

Secret symmetries and Goldstone bosons

To describe the developments that led to the salvation of gauge theories we must digress to consider the concept of a "secret symmetry", sometimes confusingly called a "spontaneously broken symmetry". This idea concerns physical systems displaying properties which seem to violate the symmetry possessed by the underlying physical laws. The best example of a secret symmetry is a permanent bar magnet. The interactions between the atoms and molecules are certainly invariant under rotations yet the bar magnet is magnetised in one particular direction. The magnet is not invariant under rotations, because the direction of magnetisation is singled out as a special one, but the underlying physical laws are. What application does this have to high energy physics? Perhaps the W bosons having a non-zero mass are like the bar magnet. Perhaps the underlying physical laws are invariant under local gauge transformations, despite the non-zero mass of the W's.

In 1961, Goldstone pointed out that any system having a secret symmetry pays a price for its secrecy: there must exist zero frequency modes or, in the case of relativistic field theory, there must exist spinless zero mass particles. These particles are called Goldstone bosons. In the ferromagnet, Goldstone bosons correspond to spin waves propagating through the solid. This would seem to spell the end of hopes for using secret symmetries in gauge theories because no zero mass Goldstone bosons have ever been observed in high energy physics experiments.

In 1964, Higgs, and independently Brout and Englert made a discovery, the true significance of which has only recently been fully appreciated. The Goldstone theorem has buried in it the assumption that there must be no long range forces; otherwise the chain of reasoning leading to zero mass Goldstone bosons breaks down. Of course, zero mass vector mesons such as the photon and the W bosons of Yang-Mills gauge theories do produce long range forces and so these theories avoid the strictures of Goldstone's theorem—Goldstone bosons need not exist.

Higgs showed that not only do zero mass Goldstone bosons not need to occur, but that a gauge theory with secret symmetry

can be made to use a spinless particle—now called the Higgs meson—to give the W bosons a mass. The freedom supplied by gauge invariance enables us to 'mix-up' the Higgs meson with a third polarisation state of the W bosons. In other words, the zero mass gauge particles which possess two states of polarisation, and the Higgs meson, which possesses one state of polarisation, combine to produce massive Ws with three states of polarisation, and the Higgs meson as an independent particle disappears. In the process the gauge symmetry seems to have been lost, but it is still there only rather well hidden. Although this fact was understood at the time, it was felt that since the symmetry is so well and truly hidden by the Higgs mechanism, there was no reason for the theory to be renormalisable.

Unified gauge theories

In 1967 two short papers appeared in which the electromagnetic and weak interactions of leptons were unified in a gauge theory using the Higgs mechanism. One by Weinberg suggested that the theory might be renormalisable; the other by Salam argued that since the underlying theory has a gauge symmetry, perhaps the Higgs mechanism does not spoil its renormalisability.

The Salam-Weinberg theory barely caused a flutter until 1971 when 't Hooft gave his non-rigorous but convincing arguments that theories of this type are indeed renormalisable. 't Hooft's work caused a considerable stir because it then became possible for a gauge theory to apply to weak interactions. Since an essential ingredient in a wide class of gauge theories is the existence of an electrically neutral W boson, extensive experimental work was initiated, not to find it directly, but to see its effects as an exchanged particle producing a weak force—as a neutral current.

Before we discuss the experimental evidence for neutral currents let us briefly describe the main features of the Salam-Weinberg theory. First we should stress that the theory originally concerned only the weak and electromagnetic interactions of leptons, the electron e^- and its neutrino ν_e , the muon μ and its neutrino ν_μ . The Salam-Weinberg theory is a gauge theory with a total of four gauge vector mesons all starting out with zero mass; but the Higgs mesons in the theory are carefully chosen to give a mass to only three of the bosons when the symmetry becomes a secret one. These are the W^+ , W^- and W^0 of the weak interactions. The fourth gauge boson remains massless and is identified with the photon. The combined existence of all four of these particles is a necessary part of the gauge symmetry itself and therefore of the renormalisability. There are ways of avoiding a neutral W boson; but the price to be paid is heavy: the number of leptons must be increased by the introduction of heavy leptons.

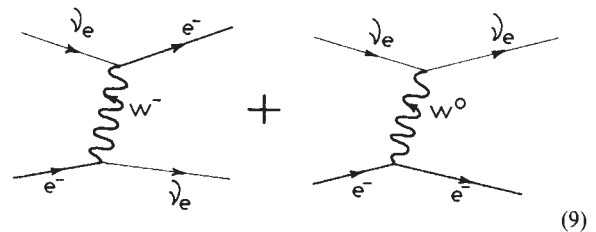
In what sense does such a gauge theory represent a unification of electromagnetic and weak interactions? This comes about through the special status that the gauge particles have in a gauge theory—they all couple to matter and to each other with the same universal coupling strength $e^2 \approx 1/137$. But whereas the exchange of zero mass particles (photons) produces an effective coupling e^2 typical of electromagnetic interactions, the exchange of W bosons of mass M_w turns out to have an effective coupling e^2/M_w^2 . For this to be as small as the observed weak interaction strength requires a very large mass M_w of at least 40 GeV, or 40 times the mass of the proton! This rather general property of unified gauge theories makes it unlikely that W bosons will be produced directly in experiments in the near future. Instead experiments have concentrated on the search for neutral current effects in neutrino scattering processes.

Evidence for leptonic neutral currents

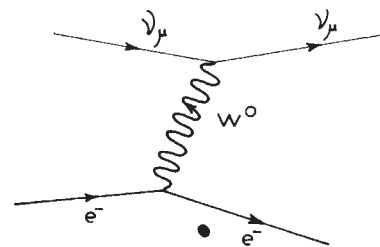
Until recently neutrino physics has been something of a poor relation in high energy physics. It is difficult to produce intense neutrino beams, and interactions are so rare that experiments take a long time to perform. In the past few years, however, with the prospect of new high energy accelerators, much effort

has been invested in new equipment, just in time to make an effective search for neutral currents.

There are only two basic processes involving leptons able to provide experimental tests for the existence of neutral currents: the scattering of either electron or muon neutrinos and anti-neutrinos off electrons. Both reactions occur at very low rates and are therefore very difficult to measure, even by the standards of neutrino physics. The first (9) would occur even in the absence of neutral currents, but the second (10) is a pure neutral current process that would be absent if only charged currents occur.

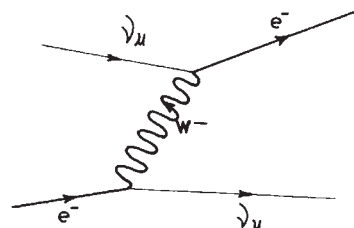


(9)



(10)

It is essential to note that (10) represents the only independent one W boson exchange diagram contributing to $\nu_\mu + e^- \rightarrow \nu_\mu + e^-$ because experiments have shown that muons and their neutrinos cannot turn into electrons and their neutrinos. These "lepton number" conservation laws are an important ingredient in the interpretation of neutral current experiments. Thus diagram (11) which would be the analogue of the first diagram in (10), is forbidden by the conservation of lepton number.



(11)

The muon neutrino experiment is the most decisive one, and was carried out at CERN using a large bubble chamber facility specifically designed for neutrino experiments. The first results were announced in 1973, showing one event believed to be $\bar{\nu}_\mu + e^- \rightarrow \bar{\nu}_\mu + e^-$. This experiment has continued to accumulate statistics, and at present the total number of neutral current events is three. It is impossible to exaggerate the difficulty of such an enterprise. Everything hinges on being certain that what is observed is not some other process. The problem is that the only observed product of the collision process is the final electron coming out of the reaction. Neither the incoming nor the outgoing neutrinos can be observed, and the electron might well result from processes involving other unobserved neutral particles such as neutrons or photons. Much of the effort and nearly all the discussion revolves around the estimation of the background events. In this experiment they have been estimated to be around 10%, but with only three events at hand who can be certain?

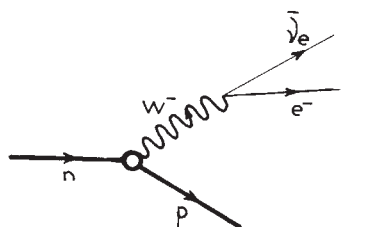
Potentially this experiment is the best test of the Salam-Weinberg gauge theory; but the most reliable evidence for neutral currents has come from neutrinos scattering off hadrons where the event rates are considerably higher. Now, however, the

strong interactions enter into the picture, to obscure and complicate the theoretical interpretation. We must consider the possible ways of extending unified gauge theories to hadrons.

Hadrons and quarks

Up to now we have been discussing the leptonic world consisting of the electron, muon and their neutrinos. As far as we know the forces between these particles are only weak and electromagnetic. Indeed, the neutrinos, being neutral, do not even have electromagnetic interactions. This is why they are able to penetrate enormous quantities of material, such as stars and the Earth itself.

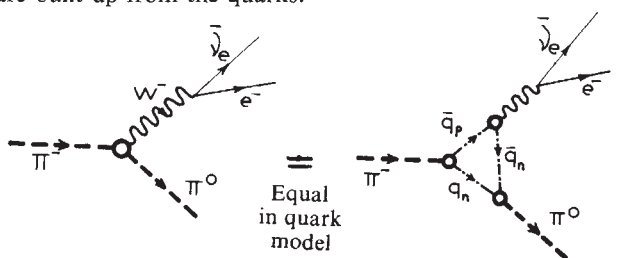
Besides the leptons there is a vast array of particles including protons, neutrons, nuclei, π mesons, K mesons, strange particles and many other so-called "elementary" particles all of which have strong or nuclear interactions. These hadrons have interactions amongst themselves some 100 times stronger than the electromagnetic interactions and some 10^{13} times stronger than the weak interactions. The structure and behaviour of hadrons is almost entirely determined by the strong interactions. Hadrons, however, also have electromagnetic and weak interactions, although these are heavily influenced by the strong interactions. An example of a hadronic weak interaction is β -decay of the neutron: $n \rightarrow p e^- \bar{\nu}_e$. How can we picture this interaction as the exchange of an intermediate vector boson? The answer, already implicit in Fermi's original theory of 1934 is that the neutron emits a charged vector boson which then decays into the leptons.



(12)

The coupling of the W boson to hadrons, however, is not as simple as it is to the leptons, because it is influenced by the strong interactions. The latter determine the structure and constitution of the hadrons, which it has become vogue to describe in terms of hypothetical fundamental constituents, the "quarks". In the quark model the hadrons are all built out of three quarks q_p, q_n, q_λ and their antiparticles $\bar{q}_p, \bar{q}_n, \bar{q}_\lambda$. This leads in a simple way to the famous SU(3) classification of particles. For our present purposes all we need note is that mesons such as the π or K are presumed to be bound states of a quark and antiquark, whereas baryons such as protons and neutrons are bound states of three quarks. Quarks have never been produced in experiments, but the remarkable success of the quark model is taken as evidence that it contains at least some element of truth.

The quark model simplifies the problem of weak decays of hadrons by enabling us to express them all in terms of the decays of only the quarks themselves. A simple example is the decay $\pi^- \rightarrow \pi^0 e^- \bar{\nu}_e$ now depicted as in (13), where the quarks are assumed to have simple weak and electromagnetic interactions like the leptons. The complications of the strong interactions are then hidden in the fashion in which the hadrons are built up from the quarks.



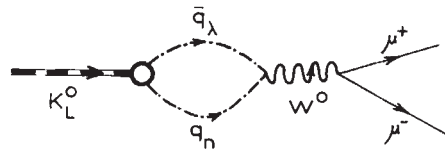
(13)

The simple symmetry properties of quarks enable them to be included in a natural elegant way in a unified gauge theory of weak, electromagnetic and possibly even strong interactions. Nearly all such theories have neutral W bosons as an essential ingredient, so that once again their exchange as neutral currents provides the best available experimental test. We shall see later that the possible existence of a new quantum number "charm" may provide even more decisive evidence in favour of unified gauge theories.

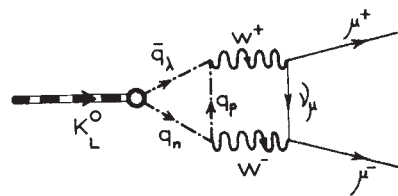
Tests for neutral hadronic currents

We have already emphasised the problematic nature of hadronic interactions; but from the point of view of gauge theories our catalogue of difficulties is by no means complete. For some years there has been powerful evidence against the existence of neutral currents in "strangeness changing" weak processes. These are processes where the initial and final hadrons have different values of the strangeness quantum number. A classic example is the strangeness changing decay $K^+ \rightarrow \pi^0 e^+ \nu_e$ which has been observed experimentally and which involves a charged current; but the very closely related decay $K^+ \rightarrow \pi^+ e^- \bar{\nu}_e$ which would involve a neutral current is suppressed by a factor of about a million.

An even more important example of a strangeness changing decay process is that of the long lived neutral K meson. This process $K_L^0 \rightarrow \mu^+ \mu^-$ involving a neutral current is 10 million times less frequent than the similar but charged current decay $K^+ \rightarrow \mu^+ \nu_\mu$. This suppression is so strong that not only must the lowest order diagram in (14) vanish, but the second order diagram in (15) also must be suppressed.

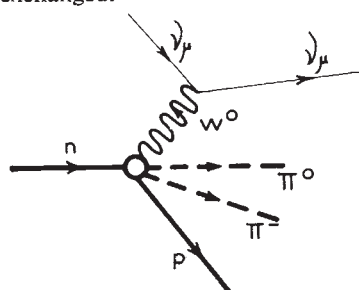


(14)



(15)

Before the advent of unified gauge theories it was assumed that this type of suppression occurred for all weak processes, whether or not there is a change of strangeness, and that neutral currents simply did not occur. Only with the renaissance of gauge theories resulting from belief in their renormalisability was it felt necessary to perform the difficult experiments required to check this suppression in hadronic processes not involving changes of strangeness. The first evidence was found in the summer of 1973 at CERN. Since that time further experiments both at CERN and in the USA have confirmed the existence of neutral currents that do not change strangeness. A typical event observed at CERN is $\nu_\mu + n \rightarrow \nu_\mu + p + \pi^- + \pi^0$. Since no strange hadrons are involved in the reaction there is no change of strangeness, but as (16) shows, there is a neutral W boson exchanged.



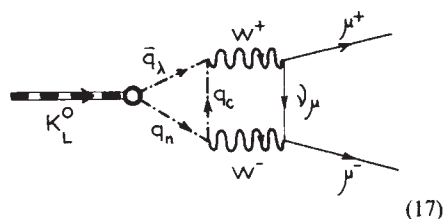
(16)

Other successful experiments have been performed which together provide conclusive evidence that neutral currents exist which do not change strangeness; but strangeness changing currents either do not exist or, if they do, are highly suppressed. What mechanism is responsible for this sharp distinction? The most elegant solution has been proposed by Glashow, Iliopoulos and Maiani.

Charm

Whenever there is a strong suppression of some otherwise innocuous process there is a good chance that a symmetry or conservation law is responsible. One way to get rid of neutral currents that change strangeness is to invoke greater symmetry so as to arrange for a universal cancellation of the unwanted processes. New symmetry means new conserved quantum numbers and new particles to carry these quantum numbers. The new quantum number invoked is "charm" and has analogous properties to those of strangeness. Just as we already have a strange quark q_s , we now need a fourth "charmed" quark q_c . It must be incorporated into a gauge theory in such a way as to preserve the gauge symmetry and therefore the vital renormalisability of the theory. In this way the unwanted process $K^+ \rightarrow \pi^+ + e^+ + e^-$ is made impossible. The unwanted $K_L^0 \rightarrow \mu^+ \mu^-$ process is, however, more subtle. The most direct way of picturing the decay shown in (14) does indeed vanish because now the W^0 cannot couple to a q_n and turn it into a q_s . But the second order diagram in Fig. 15 is still not zero.

There is now, however, an additional similar diagram involving a charmed quark in (17),



Note presence of charmed quark

which exactly cancels the diagram in (15) because of the close connection between the couplings: due to the gauge symmetry the coupling of the charmed quark q_c to W^+ and W^- is just the opposite to the proton quark q_p .

If this explanation of the absence of strangeness changing neutral currents is to be anything more than a theoretical exercise it must lead to other consequences open to experimental test. The merit of the charm theory is that it has many distinctive predictions. It invokes a new quantum number and therefore new particles carrying charm. In August 1974 M. K. Gaillard, B. W. Lee and J. L. Rosner showed how the existence of the charm quantum number would lead to an extension of the well known SU(3) classification scheme to SU(4) where the now familiar octets become groups of 15 or more particles. Most of the new particles have charm, but some do not. In particular the ϕ meson, a known and rather peculiar vector meson of mass 1.02 GeV or 1.08 times that of the proton, gains a partner, the ϕ_c whose estimated mass is about 2–3 GeV.

The new particles

In what sense is the well known ϕ meson peculiar? It has an unexpectedly long lifetime some twenty times greater than other unstable hadrons. Indeed, the strong decay into three light π mesons, $\phi \rightarrow \pi^+ + \pi^- + \pi^0$ which was expected to be its major decay mode is some $4\frac{1}{2}$ times less frequent than the decay into two heavy K mesons, $\phi \rightarrow K + \bar{K}$. The conventional explanation for the unusually long lifetime of the ϕ meson is based on the quark model. Although in principle ϕ can consist of any mixture of $q_s \bar{q}_s$, $q_p \bar{q}_p$ and $q_n \bar{q}_n$ quarks, in fact it is almost entirely made up of the strange quarks $q_s \bar{q}_s$. Pions are made up of protons and neutron quarks only, so that the ϕ meson finds it hard to turn into pions; but since ϕ is predominantly made of

strange quarks it has no difficulty manufacturing K mesons since these also contain strange quarks. The only problem for the ϕ is that the K mesons are so heavy that there is only just enough energy available to form two Ks. Thus the ϕ meson is long lived for two reasons: it is mainly composed of strange quarks, and strange K mesons are much heavier than non-strange π mesons.

The ϕ_c is a companion to the ϕ in the sense that it is expected to be mainly made of charmed quarks $q_c \bar{q}_c$ just as the ϕ is mainly composed of strange quarks. But the interesting possibility noted by Gaillard, Lee and Rosner was that the analogues of the strange K mesons, the charmed D mesons might be too heavy to be possible decay products of the ϕ_c . Thus the ϕ_c would find it difficult to decay at all and its lifetime might be even longer than that of the ϕ .

History would doubtless have consigned charm and all papers about charmed particles to that voluminous file entitled "unsuccessful higher symmetry schemes", had it not been for the discovery in November 1974 of a particle of mass 3.1 GeV and a lifetime 40 times longer than that of the ϕ ! This particle, currently named ψ , was seen both in hadron collisions at Brookhaven and in electron-positron collisions at the intersecting storage rings SPEAR in Stanford. It has been identified as a vector meson with many of the characteristics expected of the ϕ_c . But whether it is indeed the ϕ_c awaits further experimental work. Particularly interesting is the question of the existence of particles with charm, such as the D mesons. A slightly heavier long lived particle the ψ' , having a mass of 3.7 GeV was discovered soon after the ψ . The relationship between the ψ and the ψ' is playing an important part in elucidating the true nature of these new particles, as is the discovery in the same experiments of a much shorter lived particle of mass 4.1 GeV, the ψ'' . This proliferation of new particles has caused a great deal of excitement and activity. Speculations, explanations, confirmations, further facts and even candidates for charmed particles appear weekly. Sometimes the omens are good for charm: the ψ is the ϕ_c ; sometimes there is alarm as expected features are not found. At the moment of writing there are worries because no charmed decay products of the ψ'' have been seen; but until the dust settles there is no definite conclusion one can draw except that charm is still perhaps the most favoured explanation. The decisive test for charm is undoubtedly the observation of charmed particles such as the D mesons.

Unified theory of strong interactions?

At this point we have, in principle at least, a unified gauge theory of the electromagnetic and weak interactions of all elementary particles. Can we now take the ultimate step of including also the strong interactions? The problem with theories of strong interactions is that one cannot calculate their consequences accurately, since perturbation methods do not work, and so one can never test the starting hypotheses in detail.

Recently, however, there has been an important theoretical development using so called "renormalisation group" techniques, which has proved of value in the theory of phase transitions in solid state physics, and which may be of help in solving problems involving strong interactions. It has been possible to demonstrate that some types of interaction develop simpler and simpler properties as the energy increases, leading ultimately to a behaviour somewhat like that of non-interacting or free particles! Such theories are called "asymptotically free field theories", and it is possible within them to do some calculations based on perturbation methods.

Until the advent of unified gauge theories there were no known examples of asymptotically free field theories. What is quite remarkable is the discovery that a wide class of gauge theories possess this property. It may, therefore, be possible to incorporate the strong interactions into what would then be a truly unified gauge theory, and to make detailed predictions

which could then be tested experimentally. Much effort will no doubt be expended on this challenge in the next few years.

The present state of theory

We have now followed the development of gauge theories to the present day. Starting from the successful theory of quantum electrodynamics we have traced its extension into the realm of weak interactions. This led to Yang-Mills gauge theories with massive W bosons. The theories are renormalisable only because they possess an underlying symmetry which has been hidden but not destroyed by the Higgs mechanism. The most accessible experimental consequence of these theoretical ideas is the existence of neutral currents induced by neutral W boson exchange, and these have been detected in neutrino experiments. The most direct evidence for unified gauge theories would be the discovery of the W^+ , W^- and W^0 mesons; but their large predicted masses makes this an unlikely possibility for the foreseeable future. This large mass is the price paid for unifying weak and electromagnetic interactions.

The extension of these ideas to the weak and electromagnetic interactions of hadrons produced an immediate conflict with the

experimental fact that strangeness changing hadron decay processes such as $K_L^0 \rightarrow \mu^+ + \mu^-$ are highly suppressed. To achieve such suppression a new quantum number, charm, has been invoked, leading to a rich harvest of experimental predictions. The new ψ particles may indeed be indirect manifestations of this quantum number, but the final acceptance of the idea of charm depends on the success or failure of the experimental search for D mesons and other charmed particles.

The theory invoking charm is by no means unique, and failure to observe charmed particles will lead to its rapid demise. Success on the other hand will enable us to take a large step forward, providing circumstantial evidence in one fell swoop for a wide range of theoretical ideas. The stage may then be set for a grand unification of weak, electromagnetic and strong interactions. Whatever the ultimate fate of the theoretical ideas presented in this brief survey, there is no doubt that they have stimulated a remarkable series of experimental and theoretical developments. There is surely no more apposite comment than that of Einstein and Infeld some 50 years ago:

"To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks a real advance in science."

articles

Structure of the compact radio component in 3C236

E. B. Fomalount

National Radio Astronomy Observatory, Green Bank, West Virginia

G. K. Miley

Sterrewacht, Huygens Laboratorium, Leiden, The Netherlands

The compact component in the giant radio galaxy 3C236 has been studied at 0.4, 2.7 and 8.1 GHz, with resolution of up to 0.13''. The overall structure is double and asymmetric. The extent is 0.8'' in a position angle of $121 \pm 4^\circ$ which, within the uncertainties, is the same as that of the large outer 39' double. No difference between the spectra of the subcomponents was detected. On a scale of 0.2'' there is fine structure. The radio subcomponents are almost certainly not confined and are thus the results of a relatively recent event in the nucleus of 3C236. Our results are compatible with continuous flow models which are well collimated along an axis whose alignment does not change significantly during the lifetime of the source.

SMALL radio components with relatively flat spectra are now known to occur in the nuclei of many radio galaxies and QSOs. They almost certainly have an important role in producing and maintaining the extended double radio structure and their study is crucial to any understanding of the physical processes involved.

One of the most interesting examples of a double radio source with a compact central component is the giant radio galaxy 3C236. It is the only case where the radio core is sufficiently extended that its structure can be studied in detail. In 1972 Wilkinson^{1,2}, using the radio link interferometer at

Jodrell Bank, showed that the nuclear component of 3C236 (then believed to comprise the whole source) is extended by $\sim 1''$ in position angle (p.a.) 119° . Willis *et al.*³ showed that 3C236 has an overall extent of about 39' (~ 5.7 Mpc)⁴ in p.a. 122.5° . (Throughout this article a value of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is assumed for the Hubble Constant, implying a distance of 554 Mpc to 3C236.) The remarkable alignment of the $1''$ compact component with the 39' outer double has important implications for radio source models and deserves further investigation. We present here the results of a detailed study of the structure of the compact component of 3C236 at frequencies of 0.408, 2.695 and 8.085 GHz using several interferometer baselines out to a length of 950,000 wavelengths.

Observations

The 2.695 and 8.085 GHz observations were carried out with the NRAO four-element interferometer. This array consists of three steerable 85-foot antennae with a maximum baseline length of 2.7 km, and a 45-foot antenna located about 35 km south-west of the other elements. The 45-foot antenna is connected to the control building using a phase-stable radio link⁴ and three baselines of 33.1, 33.9 and 35.3 km are obtained by correlating the signals of the 45-foot element with each 85-foot antenna. The original three-element system has been described by Hogg *et al.*⁵ and Coe⁶. The noise after 15 min of observations corresponds to ~ 0.03 Jy at 2.695 GHz and ~ 0.05 Jy at 8.085 GHz. The source was observed on December 26 and 28, 1974, between sidereal times 04 h 30 min and

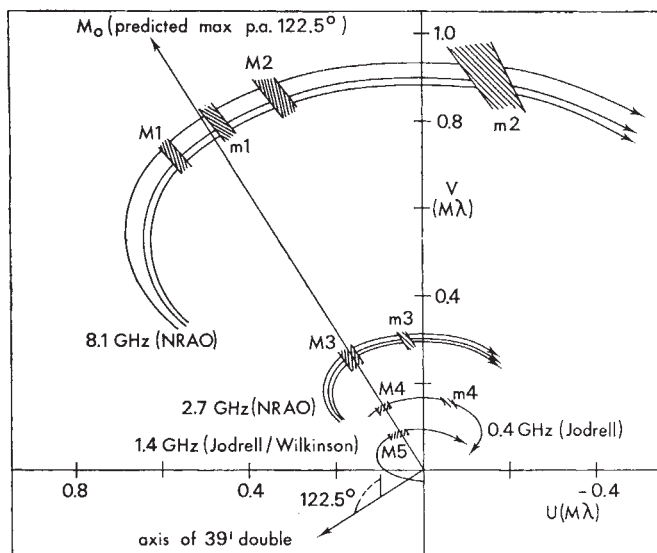


Fig. 1 The range of projected baselines used in this study. u is the east-west and v the north-south baseline in millions of wavelengths. u is measured to the left. The line denoted by M_0 indicates the amplitude maximum that would be observed from a source elongated in p.a. 122.5° (same as that of the overall 39' structure). M_1 , M_2 , M_3 and M_4 indicate observed fringe amplitude maxima and m_1 , m_2 and m_3 observed minima (see Fig. 2).

15 h 20 min. To calibrate the phase and gain drift, we alternated observations every 10 min between 3C236 and DA267, a nearby calibrator. The redundancy in the two days of observations helped us to estimate the residual phase and gain errors in the calibrated data. The baseline parameters of the array were obtained to an accuracy of $0.02''$ from subsequent observations of sources with accurately known positions (C. M. Wade and K. Johnson, unpublished). The visibility amplitude and phase of 3C236 were measured with respect to DA267 with an assumed position (epoch 1950.0) of $\alpha = 09^h 23^m 55.320^s$, $\delta = 39^\circ 15' 23.55''$ and assumed flux density (epoch 1975.0) of $S_{2695} = 4.5$ Jy and $S_{8085} = 9.7$ Jy (L. Blankenship, unpublished). The residual phase and gain errors were $\pm 10^\circ$ and $\pm 10\%$ at 2,695 MHz and $\pm 30^\circ$ and $\pm 20\%$ at 8,085 MHz and their effect was generally larger than that of the receiver noise.

The 0.408 GHz observations were made with a single baseline interferometer of length 122 km whose elements were the Mark Ia telescope at Jodrell Bank and one of the 25-m paraboloids at the Royal Radar Establishment, Defford. The system was basically similar to that described by Rowson⁷ with the addition of a digital delay line designed by B. Anderson. Here only fringe amplitudes were measured and no useful information was available about the absolute phase of the fringes.

The r.m.s. noise after a 1-min integration was equivalent to ~ 0.06 Jy. 3C236 was observed for a total of 12 h on July 19, 1974, and the system was calibrated using 3C286 (assumed unresolved and having a flux density of 22 Jy). Three simultaneous delay channels were used. One was centred on the compact source and gave fringes throughout the observing period. The others were optimised for the two 'hot spots' at the extremities of the 39' source. According to Westerbork measurements at the lower resolutions, the flux densities of these bright regions at 610 MHz are 1.8 ± 0.2 Jy and 2.1 ± 0.2 Jy respectively. We found no fringes from the hot spots larger in amplitude than 0.10 Jy, indicating that most of their flux is emitted by a region larger than $1''$.

The baseline coverage at all three frequencies is plotted in Fig. 1 together with that obtained by Wilkinson^{1,2} at 1.423 GHz. The fringe amplitude data are shown in Fig. 2.

Derivation of the radio structure

Radio maps were made at both 2.695 and 8.085 GHz by Fourier transform of the visibility data and the subsequent use of the cleaning algorithm⁸, with Gaussian restoring beams of half-power widths $0.55''$ by $0.25''$ and $0.18''$ by $0.08''$, respectively, extended in p.a. 110° . Since we know *a priori* that the compact component is smaller than $1''$ and since the NRAO array has good amplitude and phase stability, the clean procedure can be validly used here. To help judge the reliability of the cleaned maps and to interpret the data at 1.4 and 0.4 GHz where only fringe amplitude data were obtained, we fitted the fringe amplitude data at all four frequencies to various models composed of elliptical Gaussian components.

The general features of the compact component in 3C236 are displayed in the 2.7 GHz map shown in Fig. 3a. The map shows two subcomponents of unequal intensity and size. The centres are separated by $\sim 0.8''$ in p.a. $\sim 120^\circ$. The easterly component is the stronger and is not highly resolved whereas the westerly component is extended with roughly the same orientation as the outer double. In Fig. 1 the line through the origin denoted by M_0 indicates the projected baselines at which the central visibility maximum should occur for a source elongated in (and symmetrical about) p.a. 122.5° . The positions of the fringe maxima and minima observed at 2.7, 1.4 and 0.4 GHz all agree with those expected from the separation and position angles defined in the map. The parameters of the best fitting double model are given in Table 1 and the fringe amplitude function predicted by this model is shown by the solid curve in Fig. 2b. This extent and position angle agree well with

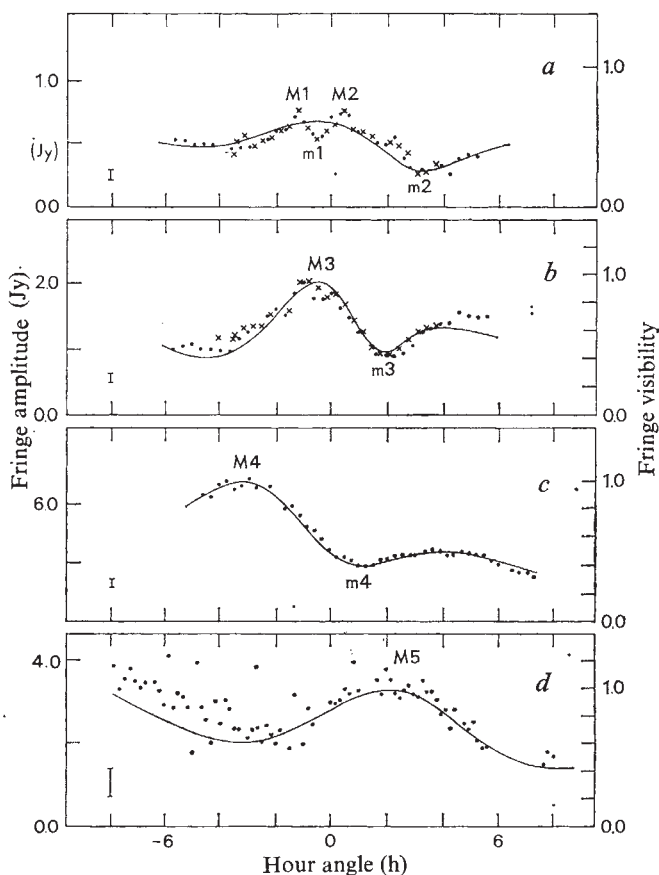


Fig. 2 Variation of fringe amplitude with hour angle at: a, 8.1 GHz; b, 2.7 GHz; c, 0.4 GHz; d, 1.4 GHz. For 8.1 and 2.7 GHz, only data from the longest of three almost equivalent baselines are shown. Bars indicate estimated total uncertainties (including systematic errors) in each fringe amplitude point. Solid lines show the fringe amplitude variations predicted by the models given in Table 1 (Fig. 2b, c, d) and Table 2 (Fig. 2a). ●, December 26; ×, December 28.

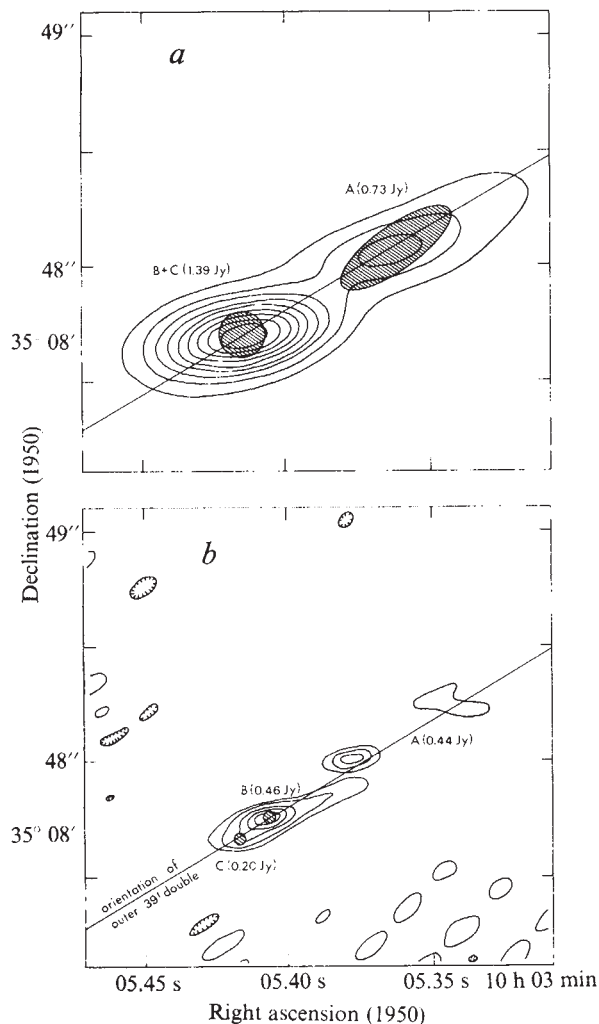


Fig. 3 Cleaned contour maps of the compact component at: *a*, 2.7 GHz with 10% contour levels; *b*, 8.1 GHz with 20% contour levels; and an additional level at 10%. The light lines at a position angle of 122.5° indicate the orientation of the 39' extended radio structure. The model brightness distribution given in Tables 1 and 2 are indicated by the shaded regions. The absolute positional accuracy is better than $0.05''$.

those previously derived from measurements at lower resolution^{1,9}.

Our observations at 0.4 GHz and those by Wilkinson at 1.4 GHz have smaller resolution than the measurements at 2.7 GHz. The fringe amplitude data are consistent with the double picture deduced from the data at 2.7 GHz. Taking the optimum 2.7-GHz model and changing only the flux densities of the subcomponents results in reasonable agreement with the observations at 0.4 and 1.4 GHz (see Fig. 2*c* and 2*d* and Table 1). There is no indication that the subcomponents have appreciably different spectra between 0.4 and 2.7 GHz.

In the case of the measurements at 8.1 GHz, the resolution is a factor of three higher and the interpretation of the structure is more complex. The easterly subcomponent dominates and is itself resolved into at least two parts denoted by B and C, separated by $0.16''$ in p.a. $121^\circ \pm 10^\circ$. This is also apparent from the deep fringe amplitude minimum m2 and the subsequent rise in the 8.1-GHz data. The optimum parameters corres-

ponding to a point double model are shown in Table 2 and the corresponding fringe amplitude variation is plotted in Fig. 2*a*. The fit is good.

Both the map and fringe data show that the westerly component A is highly resolved. Two relatively weak hot spots are present on the map at low level but are somewhat uncertain. The effect of the westerly subcomponent in the fringe amplitudes can be seen as a weak beating near transit, corresponding to the maxima M1 and M2 and the minimum m1. This beating occurs only during a limited hour angle range because of the elongated nature of this subcomponent. The line joining the origin with the central maxima M3 and M4 in Fig. 1 intersects the 8.1-GHz baseline ellipses at a position corresponding to the fringe minimum m1. Therefore, the central maximum cannot be represented as a straight line in the u - v plane. This indicates that the structure is not completely linear. The brightest regions of the $0.8''$ source define a line which is slightly rotated with respect to that of the overall emission.

We can summarise our main conclusions (see Table 3) regarding the structure of the compact source in 3C236 as follows:

- (1) The overall brightness distribution displays a double but asymmetric characteristic.
- (2) The source extent is $\sim 0.8''$ (2 kpc).
- (3) The overall position angle is $121^\circ \pm 4^\circ$.
- (4) The easterly (brighter) subcomponent is resolved into two parts, B and C, each smaller than $0.05''$ (< 130 pc) and separated by $\sim 0.16''$ in p.a. $121^\circ \pm 10^\circ$.
- (5) The westerly component A is significantly larger, elongated by $\sim 0.6''$ (1.6 kpc) along the source axis. Perpendicular to this axis the extent is $\sim 0.1''$ (0.3 kpc). There is some indication of fine structure.
- (6) On a scale of $< 0.2''$ there is structure which is not symmetrical about the overall source axis. This asymmetry limits the accuracy of conclusion (3).
- (7) Between 0.4 and 2.7 GHz there is no appreciable difference between the spectra of A and that of (B+C).

Comparison with structure of outer radio double and optical galaxy

The 39' double structure in 3C236 has a well collimated appearance with bright frontal edges or hot spots. The two outer components have roughly equal flux densities at 610 MHz, but the westerly component is less highly collimated than the easterly one and its frontal edge is well resolved at 610 MHz with the $57'' \times 99''$ beam of the Westerbork telescope. With respect to the compact nuclear component the outer edges are located at $\pm 24.1'$ in p.a. $122.7^\circ \pm 0.5^\circ$ (east) and at $-15.5'$ in p.a. $121^\circ \pm 2^\circ$ (west).

Note the following similarities between the structure of the 39' double and that of the compact nuclear component. First, the overall position angles agree to within 2° , better than the uncertainties. Second, the transverse size of the westerly structure is larger in both cases. At the position of subcomponent B the angle subtended by the westerly subcomponent A is $\sim 8^\circ$, roughly equal to the angle subtended by the outer westerly component. The radio source is identified¹¹ with a 16 mag elliptical galaxy having a redshift of 0.098. The extent of the galaxy is about $10''$ but the absolute positional accuracy of the available optical photographs is insufficient for a comparison with the radio map in Fig. 3. The galaxy is oriented in p.a. $30^\circ \pm 5^\circ$ (A. Bridle, personal communication). Within the

Table 1 Double elliptical Gaussian model derived from 2.7-GHz data

Subcomponent		Half intensity diameters		Position angle	Separation	Position angle		Flux ratios	
		Major	Minor			2.7 GHz	1.4 GHz	0.4 GHz	
West	A	$0.57''$	$< 0.20''$	$126^\circ \pm 9^\circ$	$0.77 \pm 0.04''$	$121^\circ \pm 4^\circ$	1.9 ± 0.2	1.8 ± 0.2	1.69 ± 0.15
East	B+C	$< 0.20''$	$< 0.20''$	—					

Table 2 Double point model for east subcomponent derived from 8.1-GHz data

Separation	Position angle	Flux ratio	Total flux density
$0.16 \pm 0.03''$	$121 \pm 10^\circ$	2.3 ± 0.3	0.66 ± 0.10

measurement errors the elongation of the radio structure therefore lies along the projected minor axis of the galaxy.

Discussion

First consider whether the compact component should be interpreted as (1) the relic of a past event in the nucleus which simultaneously produced the outer components of 3C236, or (2) the product of recent nuclear activity.

It is unlikely that the compact component has been contained for a period exceeding $\sim 2 \times 10^7$ yr, the light travel across the whole source. Possible confining mechanisms¹⁰ include thermal pressure, ram pressure and inertia. They must provide a confining pressure which exceeds $P_{\min}$, the minimum (equipartition) internal pressure in the subcomponents due to relativistic particles and magnetic field. For subcomponent A, $P_{\min} \sim 10^{-8}$ dyne cm^{-2} and for subcomponents B and C, $P_{\min} \sim 10^{-8}$ dyne cm^{-2} .

In the case of thermal confinement the pressure balance condition immediately yields a lower limit for the product of the density ρ and temperature T of the confining gas. Thus with $P_{\min} = 10^{-7}$ dyne cm^{-2} , $\rho T > 2 \times 10^{-5}$ g cm^{-3} degree. Now if $T \gtrsim 10^7$ K the thermal velocities within the confining gas would exceed the escape velocity of the galaxy. Such a hot

the relativistic plasma is expelled must have remained constant to better than 4° over more than 2×10^7 yr. It is presumably the rotational axis of the galaxy or of some dense region inside its nucleus. Aligned recurrent expulsions are difficult to reconcile with the 'gravitational slingshot' model of Saslaw *et al.*¹⁴ in which massive objects are flung out in the rotation plane of the elliptical galaxy. Furthermore, the alignment of the radio components along the minor axis of the galaxy provides an additional argument against this mechanism.

One class of models requiring quasi-continuous nuclear activity, which recently received considerable attention, involves the continuous flow of energy from the nucleus to hot spots in the outer components of double radio sources¹⁵⁻¹⁷. This energy is efficiently transported in a narrow beam at relativistic speeds. All the schemes that have been proposed to produce such relativistic beams (rotating massive object¹⁵, Laval nozzles¹⁷ and so on) operate over distances $\lesssim 100$ pc. It is therefore unlikely that the entire 2 kpc 'compact' component of 3C236 could be associated with the production of the beam. But the radio emission could well be due to interaction of the beam with non-relativistic gas in the vicinity of the nucleus of the galaxy. A fraction of the bulk energy in the beam would then be randomised in the galaxy rather than in the outer regions of the beam and the galaxy would seem to 'glow' at radio wavelengths. In this case the detailed radio structure would merely reflect that of the entrained non-relativistic gas illuminated by the relativistic beam. Such a picture would be consistent with the remarkable alignment, the agreement between the angles subtended by the westerly 'cone of ejection'

Table 3 The major subcomponents in the radio nucleus of 3C236

	Half intensity diameters		Position angle	Position*		Flux densities (Jy)				Spectral index
	Major	Minor		East	North	8.1 GHz	2.7 GHz	1.4 GHz	0.4 GHz	
A	$\sim 0.6''$	$\sim 0.1''$	$126 \pm 9^\circ$	$-0.43 \pm 0.03''$	$+0.26 \pm 0.03''$					
B	$< 0.05''$	$< 0.05''$		$0.12 \pm 0.02''$	$-0.08 \pm 0.02''$	0.44 ± 0.10	0.73 ± 0.08	1.15 ± 0.15	2.6 ± 0.15	0.67 ± 0.05
C	$< 0.05''$	$< 0.05''$	$121 \pm 10^\circ$	$0.25 \pm 0.02''$	$-0.16 \pm 0.02''$	0.46 ± 0.05				
Total	$0.8'' \pm 0.1''$	$0.1''$	$121 \pm 4^\circ$	0.0	0.0	1.39 ± 0.10	2.05 ± 0.20	4.4 ± 0.20		0.61 ± 0.05
Outer double	$2,340''$	$60''$	122.5 ± 0.5			0.20 ± 0.05	2.12 ± 0.04	3.2 ± 0.2	7.0 ± 0.3	0.65 ± 0.05

*Position is given with respect to 2.7-GHz centroid (epoch 1950) $\alpha = 10^h 03^m 05.396 \pm 0.003$ s, $\delta = 35^\circ 08' 47.84 \pm 0.03''$.

gas could not be maintained within the galaxy. An additional argument for even lower temperatures is provided by the presence of the [O II] $\lambda 3727$ line in the optical spectrum of 3C236 (refs 11 and 12). Taking $T < 10^7$ K gives $\rho > 2 \times 10^{-22}$ g cm^{-3} . Since the confining gas must be present over distances of at least 2 kpc ($\sim 0.7''$), a total mass of $> 10^{11} M_\odot$ would be required. Also, such large densities, with a magnetic field strength comparable to the equipartition value of $\sim 4 \times 10^{-4}$ gauss, would result in Faraday rotation of $\sim 10^8$ rad and complete depolarisation of the source at centimetre wavelengths. Since this component has been found to be polarised by $\sim 2\%$ at 6 cm (ref. 13 and Baker, unpublished) we discount the possibility of thermal confinement.

Ram pressure confinement would need even higher gas densities. Scaling a velocity $< c$ for the outer components to the compact central component results in an expansion velocity $v < 100$ km s^{-1} . Since $\rho v^2 > 10^{-7}$ dyne cm^{-2} , $\rho \gtrsim 10^{-21}$ g cm^{-3} for confinement.

At first sight inertial confinement by a relatively cold gas inside the subcomponents seems more promising. Again taking $v < 100$ km s^{-1} , this gas must have a total mass $> 2 \times 10^8 M_\odot$, corresponding to a density $\gtrsim 5 \times 10^{-25}$ g cm^{-3} . Since, for these densities also no polarisation should be measurable, inertia cannot provide the confining pressure. We have therefore shown that the compact central component of 3C236 is not confined and is unlikely to be a remnant of the event which gave birth to the large double radio source.

It is almost certainly the result of a relatively recent outburst in the nucleus of the galaxy. In this case, the axis along which

in the compact and extended structures and the observed asymmetry.

A more detailed picture of the compact component requires sensitive observations with even higher resolution. Measurements with VLBI techniques may reveal whether any of the subcomponents discussed here have even smaller scale features. Structure on a scale of $\lesssim 0.01''$ might relate to the origin of the radio event. In addition, an intensive optical investigation of 3C236 should be made. Photographs with a positional accuracy of $0.1''$ could be usefully compared with our radio data. Together with a spectroscopic study of the distribution of [O II] $\lambda 3727$ such observations should enable a clearer picture to be obtained of the dynamics and physical conditions within this remarkable galaxy.

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¹ Wilkinson, P. N., *Mon. Not. R. astr. Soc.*, **160**, 305 (1972).

² Wilkinson, P. N., *Ann. Jodrell Bank*, **2**, 58 (1973).

³ Willis, A. G., Strom, R. G., and Wilson, A. S., *Nature*, **250**, 625 (1974).

⁴ Sarma, N. V. G., *Electronics Internal Report No. 126*, (NRAO, Green Bank, 1973).

⁵ Hogg, D. E., Macdonald, G. H., Conway, R. G., and Wade, C. M., *Astr. J.*, **74**, 1206 (1969).

⁶ Coe, J. R., *Proc. IEE*, **61**, 1335 (1973).

- ⁷ Rowson, B., *Ann. Jodrell Bank*, **2**, 2 (1973).
⁸ Högbom, J. A., *Astr. Astrophys. Suppl.*, **15**, 417 (1974).
⁹ Pooley, G. G., and Henbest, S. N., *Mon. Not. R. astr. Soc.*, **169**, 477 (1974).
¹⁰ Longair, M. S., Ryle, M., and Scheuer, P. A. G., *Mon. Not. R. astr. Soc.*, **164**, 243 (1973).
¹¹ Sandage, A., *Astrophys. J. Lett.*, **150**, L145 (1967).

- ¹² Matthews, W. G., and Baker, J. C., *Astrophys. J.*, **170**, 241 (1971).
¹³ Wright, W. E., thesis, California Institute of Technology, **2**, 68 (1973).
¹⁴ Saslaw, W. C., Valtonen, M. J., and Aarseth, S. J., *Astrophys. J.*, **190**, 253 (1974).
¹⁵ Rees, M. J., *Nature*, **229**, 312 (1971) (erratum p.510).
¹⁶ Scheuer, P. A. G., *Mon. Not. R. astr. Soc.*, **166**, 513 (1974).
¹⁷ Blandford, R. D., and Rees, M. J., *Mon. Not. R. astr. Soc.*, **169**, 395 (1974).

Ligand-induced redistribution of lymphocyte membrane ganglioside GM1

Thomas Révész* & Melvyn Greaves

ICRF Tumour Immunology Unit, Zoology Department, University College, London WC1E 6BT, UK

Dynamic aspects of the binding of cholera toxin to lymphocyte membranes have been studied. We have shown that the receptor for this ligand—the GM1 ganglioside—can be laterally redistributed into aggregates and caps. Exogenous purified GM1 inserted into GM1-deficient human leukaemic cells can undergo a similar pattern of ligand-induced mobilisation. These observations may have important implications for both the general behaviour of cell surface glycolipids and the mode of action of cholera toxin on adenyl cyclase.

INTERACTION of divalent or polyvalent ligands (for example, antibodies, lectins) with cell surface structures on lymphocytes and many other cell types induces redistribution of the largely diffuse binding sites into clusters and into highly polarised single aggregates or 'caps'^{1,2}. The capping process is not fully understood but depends on active cellular metabolism and is probably regulated by microfilaments and microtubules³. These observations along with species antigen intermixing in mouse-man heterokaryons² provide visually dramatic evidence for a fluid mosaic model of cell membranes⁴. Lateral mobility of both proteins and lipids (which have similar diffusion constants) has also been demonstrated amply by electron spin resonance and electron magnetic resonance studies (reviewed in ref. 2).

A principal implication of membrane fluidity is clearly that translational movement induced by physiological ligands may facilitate molecular interactions that play a critical role in signal transduction across membranes.

Lipids may play a crucial role in cell surface receptor function by regulating molecular mobility and also by virtue of selective hydrophobic association with integral membrane proteins^{5,6}. The marked deficiency of several glycolipids, particularly the charged gangliosides, in virus and carcinogen-transformed animal cells⁷, in human leukaemia cells⁸, and in normal cells at particular phases of the cell cycle⁹, suggests that these lipids have an important role in growth control.

The ganglioside GM1 (monosialo-gangliotetraosyl-ceramide) is the natural receptor for cholera toxin¹⁰⁻¹² and it is now well established that cholera toxin (cholera toxin) exerts both its clinical effects (intestinal hypersecretion and diarrhoea) and its experimental effects (for example, induced steroidogenesis, inhibition of proliferation) through the activation of adenylate cyclase and accumulation of cyclic AMP¹³⁻¹⁵. Cuatrecasas and colleagues have proposed that lateral diffusion of cholera toxin-GM1 complexes within the plane of the membrane may lead to interaction with adenylate cyclase and that GM1 might play an important role not only in general growth regulation

but also in the coupling of hormone-induced responses¹⁶. Similarly, Field has suggested that cholera toxin may (through GM1) stabilise a catecholamine-sensitive conformation of adenylate cyclase in the turkey erythrocyte membrane¹⁷.

So far no demonstration of ligand-induced redistribution of a glycolipid has been reported. In view of the intriguing relationship between GM1 and adenylate cyclase it would be of considerable interest to determine conditions for redistribution of this glycolipid into aggregates and caps. A comparison of ganglioside and glycoprotein redistribution might reveal important implications for functional associations of gangliosides and proteins and information of relevance to signal transduction mechanisms in membranes. We demonstrate here, using cholera toxin and labelled antibodies as ligands, that GM1 on lymphocytes can indeed be redistributed into aggregates and caps, and moreover that GM1 inserted into GM1-deficient cells undergoes a similar pattern of ligand-induced redistribution.

Capping of lymphocyte membrane ganglioside

The experimental system for visualising the binding of cholera toxin to the lymphocyte surface is a three-layer immunofluorescence procedure (see legend to Fig. 2). Observations were performed using a Zeiss ultraviolet microscope with Plume, incident illumination and also the fluorescence activated cell sorter (FACS-1, Becton Dickinson, Mountain View, California)^{18,19}.

All lymphocytes, monocytes and granulocytes studied bound cholera toxin to their surface and uptake was rapidly saturated at 20 °C. Binding was inhibited by purified GM1 (Fig. 1). The biologically inactive cholera toxin bound almost as effectively as cholera toxin—as shown in other cell systems and expected from its competitive antagonistic capacity. At excess cholera toxin concentration there was no apparent difference between thymocytes, T and B lymphocytes; however, murine lymphocytes bound considerably more cholera toxin than human cells. We have shown previously that cholera toxin inhibits the proliferation *in vitro* of mouse T and B lymphocytes and L1210 lymphoma cells within a concentration range of 10⁻³–10⁻⁴ µg ml⁻¹ (ref. 8). The proliferation of human peripheral lymphocytes, however, was enhanced by these doses of cholera toxin, and only higher doses inhibited their mitogen-induced proliferative response.

Figure 2 illustrates the pattern of GM1 distribution observed on mouse and human cells incubated with different concentrations of cholera toxin. When all sequential steps were performed at 4 °C or at room temperature in the presence of 0.2 M sodium azide the distribution of fluorescence was either in aggregates of various sizes or, at higher concentrations (particularly on mouse cells), as intense rings. When all steps were performed at 37 °C on human lymphocytes very little surface fluorescence was detectable. We interpreted this observation as indicating that cholera toxin had been internalised rapidly or shed, and this view was supported by the following observations. When

*Permanent address: Second Department of Paediatrics, Semmelweis University of Medicine, Budapest, Hungary.

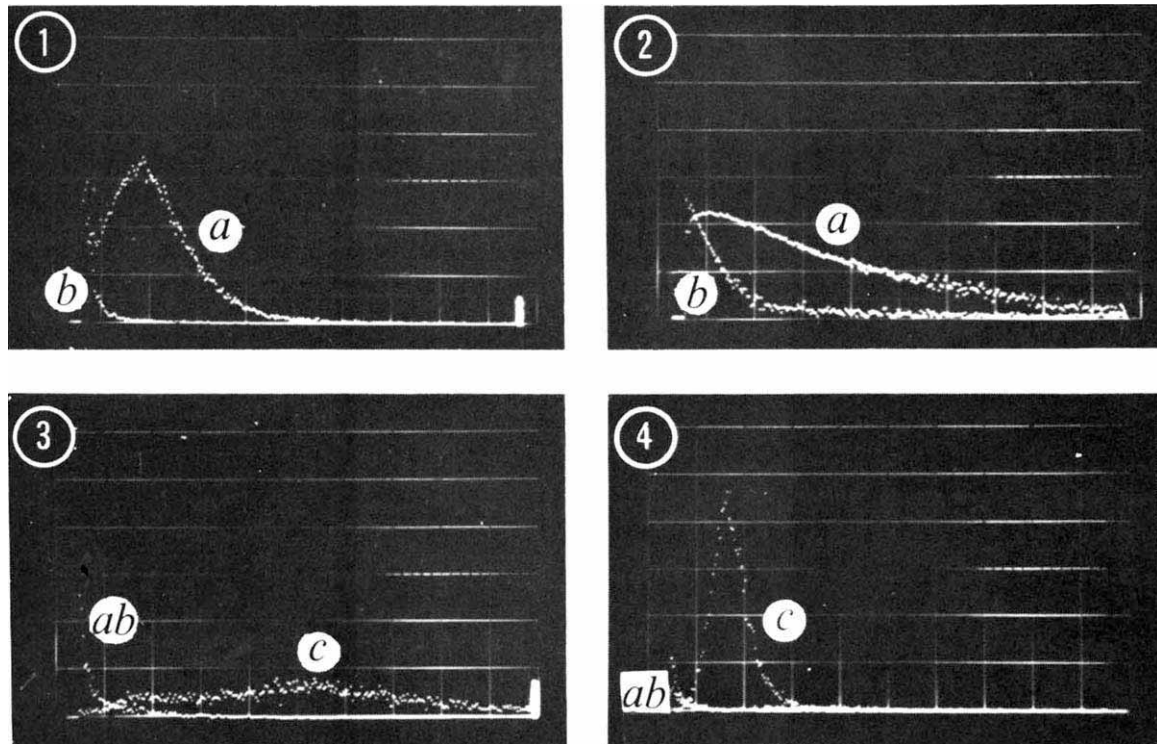


Fig. 1 Fluorescence activated cell sorter (FACS) analysis of cholera toxin binding to normal lymphocytes and leukaemic cells. The essentials of the immunofluorescence method are outlined in the legend for Fig. 2. Abscissa, relative fluorescence intensity; ordinate, relative number of cells, 10,000 cells counted in each sample. 1, Human thymus lymphocytes: *a*, cholera toxin; *b*, control (horse anti-choleragenoid and rabbit anti-horse- γ -fluorescein-isothiocyanate only). 2, Human tonsil lymphocytes: *a*, cholera toxin; *b*, cholera toxin preincubated with purified GM1 ganglioside. 3, Acute myeloblastic leukaemia cells: *a*, and *b* (superimposed), as in 1; *c*, cholera toxin binding after pretreatment of AML cells with neuraminidase (10 IU ml^{-1}). 4, Acute lymphoblastic leukaemia cells: *a*, and *b* (superimposed), as in 1; *c*, cholera toxin binding to cells that have incorporated exogenous GM1 (purified from brain—gifts from Drs R. Murrey and D. Critchley) into their surface membrane.

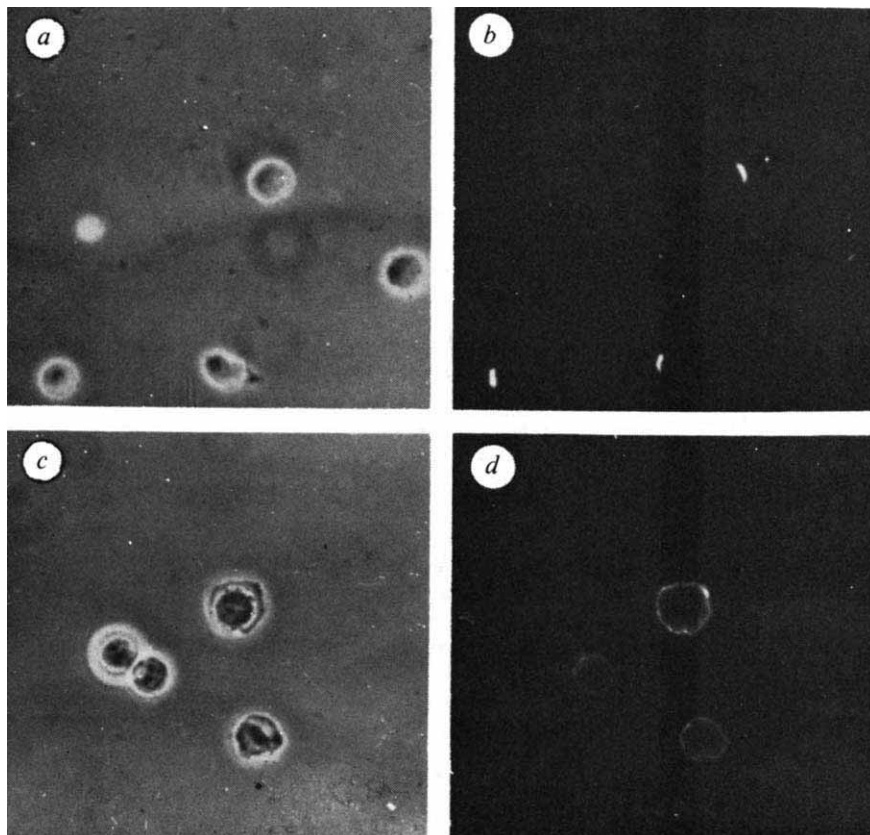


Fig. 2 Surface fluorescence pattern of human and murine lymphocytes labelled with cholera toxin. Cells in 0.2 ml Earl's saline ($2 \times 10^7 \text{ ml}^{-1}$) were incubated with cholera toxin ($100 \mu\text{g ml}^{-1}$) for 10 min at 4°C . After washing the cell suspensions twice, horse anti-choleragenoid serum was added in a final dilution of 1:10, and the cells were incubated at 21°C for 30 min. Further washing was followed by a 1:10 dilution of rabbit anti-horse- γ -globulin-fluorescein, isothiocyanate, and incubation at 37°C for 30 min. Washed cell suspensions were mounted on slides and examined using a Zeiss Ultraphot II microscope modified for epifluorescence microscopy. Photographs were taken with a Pentax camera. ($\times 524$). *a*, Human tonsil cells (phase contrast); *b*, human tonsil cells (fluorescence); *c*, murine spleen cells (phase contrast); *d*, murine spleen cells (fluorescence).

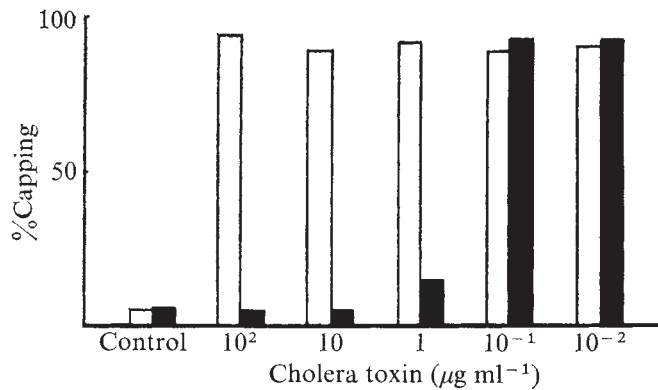


Fig. 3 Capping of cholera toxin on human and murine thymocytes. Lymphocytes in 0.2 ml Earl's saline (2×10^7 ml $^{-1}$) were incubated with various doses of cholera toxin for 10 min at 4 °C. Horse anti-cholera toxin was added to give a final concentration of 1:10, and cell suspensions were incubated at 21 °C for 30 min. The third incubation—with rabbit anti-horse- γ -fluorescein isothiocyanate was carried out at 37 °C for 30 min. Caps were defined as cell surface fluorescence restricted to one third or less of the cell surface. Open columns, human; black columns, mouse.

cholera toxin was added at 4 °C and either or both of the antibody layers at 37 °C, intense surface fluorescence was visible. The pattern of fluorescence as shown in Fig. 3 depended on the concentration of cholera toxin used and the species origin of lymphocytes. Human lymphocytes showed pronounced redistribution into discrete caps over a wide range of concentrations (compare Fig. 2) in contrast, mouse lymphocytes only showed caps with the lower concentrations used. Significantly, the biologically inactive cholera toxin gave essentially the same results as cholera toxin. By incubating mouse spleen lymphocytes with low concentrations (10^{-4} – 10^{-2} μg ml $^{-1}$) of cholera toxin at 37 °C followed by the two antibody layers at 4 °C in the presence of azide it was possible to demonstrate capping on approximately 50% of reactive cells. Although the intensity of cap fluorescence was less than when the second and/or third steps were performed at higher temperatures (compare Fig. 2), this result demonstrated that cholera toxin itself can induce redistribution into caps. We suspect that the lack of observable capping of GM1 on mouse lymphocytes treated with high concentrations of cholera toxin is related to a higher level of GM1 in these cells compared with human lymphocytes. Although we have not yet quantified cholera toxin binding sites on these cells, this interpretation is supported by the observation that capping of GM1 on human cells (at high cholera toxin concentrations) is inhibited by creation of more GM1 binding sites for cholera toxin. This was achieved both by neuraminidase pretreatment—which converts other gangliosides (that is GD1a, GT1; di- and tri-sialo-gangliosides) into GM1 by cleaving off sialic acid residues²⁰—and by direct insertion of GM1 into cells (Table 1).

Table 1 Inhibition of capping of GM1 on human tonsil cells

Pretreatment	% Capping
None	89.5
Trypsin (0.05%)	85.0
Neuraminidase (10 IU)	2.2
GM1 insertion (20μg)	3.0
Colchicine (10^{-4} M)	86.5
Cytochalasin B (10 μg)	48.2
Colchicine (10^{-4} M)+ cytochalasin B (10 μg)	27.6

Human tonsil cells (2×10^7 ml $^{-1}$) were incubated with trypsin, neuraminidase and purified GM1 at 37 °C for 30 min followed by cholera toxin at 4 °C, horse anticholera toxin at 21 °C and rabbit anti-horse- γ -globulin-fluorescein-isothiocyanate at 37 °C. Colchicine and cytochalasin B were added to the appropriate cell suspensions 5 min before each step. Caps were defined as cell surface fluorescence restricted to less than one third of the cell surface.

Holmgren and colleagues recently reported on the distribution of mouse thymus lymphocyte GM1 using a triple layer system essentially the same as that reported here²¹. They observed both ring staining and aggregation, but in contrast to our results, no cap formation. The reported concentration of cholera toxin used in these experiments, however, was 1 μg ml $^{-1}$. As can be seen from Fig. 3, this dose is considerably above the maximal concentration for cap induction, in mouse lymphocytes.

Capping of immunoglobulin molecules on lymphocytes is inhibited by a combination of colchicine and cytochalasin^{22,23}. Although these drugs may have diverse effects on cells, these studies suggest that microfilaments and microtubules have an important role in molecular mobility in the membrane³. Significantly, these drugs have precisely the same effect on GM1 redistribution (Table 1); in combination they are more potent than either alone. Although GM1 is the biologically relevant receptor for cholera toxin and free GM1 can completely inhibit binding to cells (see above and refs 11 and 12), cholera toxin does bind at least weakly to other gangliosides and also to some glycoproteins. It was therefore theoretically possible that the capping we observed was not due to GM1 redistribution but to movement of other structures. The following experiment provides, however, an unequivocal demonstration of ganglioside redistribution into caps.

Gangliosides inserted into membranes

Many transformed animal cell lines are ganglioside deficient⁷, and we have reported that virtually all human acute leukaemia cells have a considerable deficiency in binding sites for cholera toxin⁸. These cells efficiently incorporate exogenous GM1 into

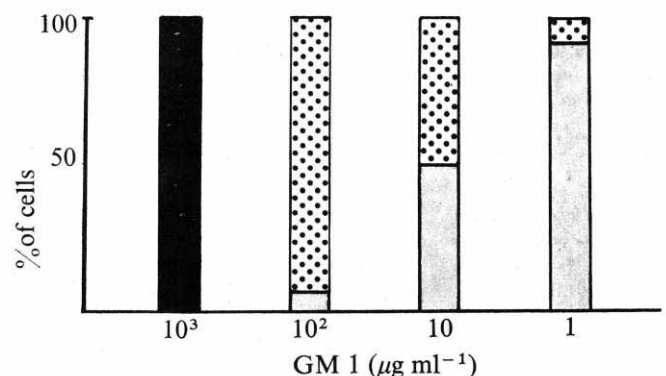


Fig. 4 Insertion and capping of purified GM1 in human acute lymphoid leukaemia cells. Leukaemia cells in 0.2 ml Earl's saline (2×10^7 ml $^{-1}$) were incubated with various doses of purified GM1 for 30 min at 37 °C. After washing the cells twice in Earl's saline cholera toxin (100 μg ml $^{-1}$) was added, followed by two antisera, as described in the legend for Fig. 2. Fluorescence staining patterns: Solid columns=ring (continuous surface staining—see Fig. 2); dotted columns=patchy (fluorescence aggregates of various sizes), stippled columns=caps (surface fluorescence restricted to less than one-third of the cell surface).

their membranes and bind cholera toxin^{8,24} (Fig. 1); alternatively, if they are treated with neuraminidase, cholera toxin binding occurs, presumably by the conversion of other gangliosides to GM1 (Fig. 1). We have performed similar experiments with the rodent cell lines BHK and NIL; however, because these cells are by chemical criteria deficient in all gangliosides²⁵ they can be rendered cholera toxin-reactive by GM1 insertion but not by neuraminidase treatment. After either GM1 insertion or neuraminidase treatment acute leukaemia cells show pronounced capping provided that not too much GM1 is inserted (Fig. 4); a result predicted by our earlier observations (compare Fig. 3). Although it is theoretically possible that GM1 insertion reveals endogenous non-GM1 binding sites for cholera toxin,

we regard this as extremely unlikely and therefore suppose that all the redistribution we observe is due solely to lateral mobilisation of the inserted GM1. These experiments provide compelling evidence for the lateral redistribution of glycolipids.

Possible mechanisms of glycolipid redistribution

The mechanics and molecular requirements for glycolipid redistribution are of course unknown. It is however possible to conceive of two basically different means by which a glycolipid such as GM1 might become capped. It may do so by association with integral proteins or a particular protein. Alternatively, glycolipids on motile lymphocytes might be in continual orientated motion, cross linkage by ligands then resulting in 'trapping' at the rear 'pole' of the cell. At present we have no unequivocal evidence which clearly supports either of these alternatives; distinguishing between them will perhaps have important implications for the dynamics and functional roles of lipids in cell membranes. The capping process is insensitive to trypsin (Table 1) and so we assume that if GM1 did have an obligatory association with an integral protein then the latter structure is trypsin insensitive—at least as far as its facilitative interaction with GM1 is concerned.

Since exogenous GM1 molecules inserted into membranes can be redistributed instantly we must also assume that any specific conditions necessary for mobilisation would have to be established rapidly by newly incorporated molecules. It is particularly relevant in this respect that at least some of the GM1 molecules and other glycosphingolipids incorporated into the membrane of glycolipid-deficient transformed cells can alter growth characteristics²⁶ and render cells sensitive to the biological effect of cholera toxin²⁴. The aggregation of GM1 molecules into clusters and caps which is induced by 'physiological' concentrations of cholera toxin may be very relevant to the regulation of adenylate cyclase activity as suggested by Cuatrecasas and colleagues. But since the biologically inactive cholera toxinoid has virtually the same binding capacity as cholera toxin and can also induce redistribution of GM1 (see

above) it would seem that aggregation of GM1 molecules is not in itself sufficient to alter adenylate cyclase activity.

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- ¹ Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225–229 (1971).
- ² Edidin, M., *A. Rev. Biophys. Bioengng*, **3**, 179–201 (1974).
- ³ Edelman, G. M., Yahara, I., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1442–1446 (1973).
- ⁴ Singer, S. J., and Nicholson, G. L., *Science*, **175**, 720–731 (1972).
- ⁵ Birnbaumer, L., *Biochim. biophys. Acta*, **300**, 129–158 (1973).
- ⁶ Rodbell, M., *Current Topics in Biochemistry* (edit by Anfinsen, C. B., Goldberger, R. F., and Schechter, A. N.), 187–218 (Academic, New York, 1972).
- ⁷ Hakomori, S., *Biochim. biophys. Acta*, **417**, 55–89 (1975).
- ⁸ Revesz, T., and Greaves, M. F., in *Int. Symp. Membrane Receptors of Lymphocytes* (edit. by Seligmann, M., Preud'homme, J. L., and Kourilsky I.) (ASP Biological and Medical Press, Amsterdam, in the press).
- ⁹ Gahmberg, C. G., and Hakomori, S., *Biochem. biophys. Res. Commun.*, **59**, 283–291 (1974).
- ¹⁰ King, C. A., and van Heyningen, W. E., *J. infect. Dis.*, **127**, 639–647 (1973).
- ¹¹ Holmgren, J., Lonnroth, I., and Svennerholm, L., *Scand. J. infect. Dis.*, **5**, 77–78 (1973).
- ¹² Cuatrecasas, P., *Biochemistry*, **12**, 3547–3558 (1973).
- ¹³ Pierce, N. F., Greenough, W. B. III, and Carpenter, C. C., *Bact. Rev.*, **35**, 1–13 (1971).
- ¹⁴ Field, M., *New Engl. J. Med.*, **284**, 1137–1144 (1971).
- ¹⁵ Finkelstein, R. A., *Cholera, CRC Crit. Rev. Microbiol.*, **2**, 553–623 (1973).
- ¹⁶ Bennett, V., O'Keefe, E., and Cuatrecasas, P., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 33–37 (1975).
- ¹⁷ Field, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3299–3303 (1974).
- ¹⁸ Bonner, W. A., Hulett, H. R., Sweet, R. G., and Herzenberg, L. A., *Rev. Scient. Instrum.*, **43**, 404–409 (1972).
- ¹⁹ Hulett, H. R., Bonner, W. A., Sweet, R. G., and Herzenberg, L. A., *Clin. Chem.*, **19**, 813–816 (1973).
- ²⁰ Kuhn, R., Wiegandt, H., and Egge, H., *Angew. Chem.*, **73**, 580–581, (1961).
- ²¹ Holmgren, J., Lindholm, L., and Lonnroth, I., *J. exp. Med.*, **139**, 801–819 (1974).
- ²² Unanue, E. R., and Karnovsky, M. J., *J. exp. Med.*, **140**, 1207–1220 (1974).
- ²³ De Petris, S., *Nature*, **250**, 54–56 (1974).
- ²⁴ Cuatrecasas, P., *Biochemistry*, **12**, 3558–3566 (1973).
- ²⁵ Critchley, D. R., and Macpherson, I., *Biochim. Biophys. Acta*, **296**, 145–159 (1973).
- ²⁶ Laine, R. A., and Hakomori, S., *Biochem. biophys. Res. Commun.*, **54**, 1039–1045 (1973).

Genetic perturbations that reveal tertiary conformation of tRNA precursor molecules

William H. McClain & J. G. Seidman

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

The ubiquity of tRNA-like conformations in tRNA precursors of prokaryotes provides a common structural basis for precursor recognition by the maturation enzymes. This design eliminates the need for multiple enzymes to achieve the maturation of different precursors. Moreover, the requirement of this specific conformation for maturation guards against the production of potentially deleterious mutant tRNAs.

THE salient features of tRNA biosynthesis in prokaryotic organisms are fairly well understood. The process begins by transcription of a tRNA gene to yield a large precursor RNA, which is then converted into tRNA by a series of enzymatic steps that include the modification of specific nucleotides and cleavage of the precursor RNA. The absence of inaccurately processed precursor RNAs in normal cells demonstrates the accuracy of this multi-step process. Such specificity must be determined in part by precursor RNA conformation, so it is at this level of complexity that we must investigate the mole-

cular mechanism that guarantees accurate tRNA synthesis. Precursor RNA conformation and its relation to tRNA biosynthesis are the subjects of this report.

The original observation on which our experiments were based is that mutationally altered forms of phage T4 glutamine tRNA contain reduced levels of modified nucleotides¹. Since modification depends on conformation, these mutants might be used for indirect study of precursor RNA conformation. Modified nucleotides are formed at the level of precursor RNA by enzymatic modification of one of the standard nucleotides. Comparison of many tRNA sequences indicates that the formation of modified nucleotides rarely if ever involves enzymatic recognition of the primary nucleotide sequence alone². These considerations and other kinds of evidence³ indicate that recognition relies on the secondary and possibly tertiary conformations of the RNA molecules. The lack of modified nucleotides in mutant glutamine tRNAs therefore implies the existence of distorted conformations of the corresponding precursor RNAs, which contain glutamine and leucine tRNA sequences^{4,5}. These considerations raise an interesting question. Are deficiencies in nucleotide modifications

confined to the glutamine tRNA region of the precursor RNA or are they also present in leucine tRNA? This obviously will depend on the specific conformation of the precursor RNA at the time its nucleotide residues are modified, as illustrated by the following example. If there is little or no interaction between the residues of the two tRNA sequences, as would be the case with two cloverleaf secondary structures, then only glutamine tRNA will be deficient in nucleotide modifications. By contrast, if there are extensive interactions between the residues of the two tRNA sequences, then the modified residues of both tRNAs will be deficient in the mutants. We have tested these possibilities by isolating mutant precursor RNAs and determining their content of modified nucleotides. As viewed by this kind of analysis the conformation of the precursor RNA involves little or no interaction between the residues of the two tRNA sequences.

Mutant-specific patterns

Precursor RNAs labelled with ^{32}P were purified to more than 80% radiochemical homogeneity by two successive steps of polyacrylamide gel electrophoresis. They were then analysed for modified nucleotides. Table 1 gives the percentages of modification at twelve positions in the glutamine-leucine precursor RNA that is synthesised after infection of *E. coli* by the *psu*₂⁻ ochre-suppressing strain of phage T4. *psu*₂⁺ is the structural gene of an ochre-suppressing glutamine tRNA¹. A value of 100% in

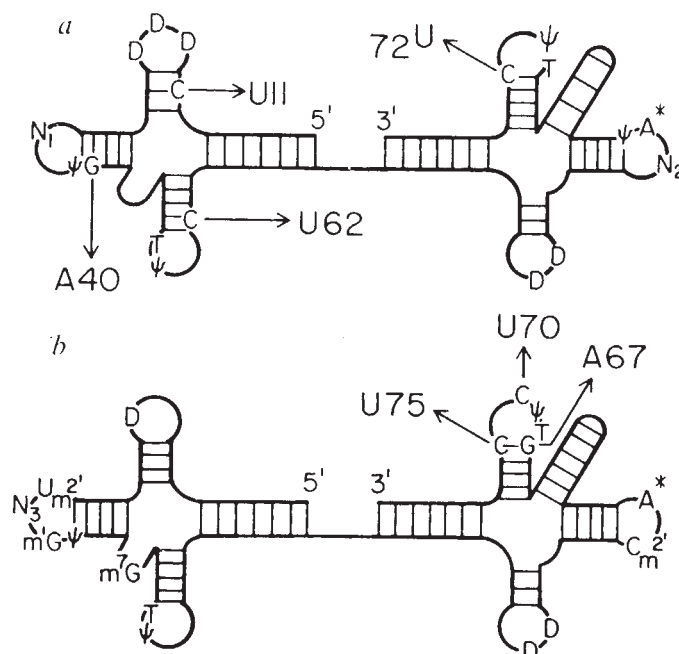


Fig. 1 Positions of modified and mutant nucleotides in glutamine-leucine (a) and proline-serine (b) precursor RNAs. Other residues are omitted. The phosphate-sugar backbone of the tRNA sequences is designated by the thick continuous line that is arranged in a double cloverleaf conformation. Hydrogen bonds involved in maintaining the cloverleaf conformations are designated by the short thin lines that are parallel. The 5' to 3' polarity of the RNA chains is indicated. Residue N₁ at the top left is in the anticodon of the *psu*₂⁺ glutamine tRNA sequence¹, and N₂ on the right is in the anticodon of leucine tRNA¹⁷. At the bottom left N₃ is in the anticodon of the proline tRNA sequence^{6,7}, whereas Cm^{2'} and A* bracket the anticodon of the *psu*₁⁺ serine tRNA^{6,9}. Arrows show nucleotide substitutions in the individual mutant of *psu*₂⁺ (top) or *psu*₁⁺ (bottom). All mutants have been described previously^{1,8,9} except for 72U, a newly-isolated mutant of *psu*₂⁺ (our unpublished results).

Table 1 Percentage of nucleotides that are modified in glutamine-leucine precursor RNAs

Glutamine tRNA nucleotide (position)	RNase fragment analysed	<i>psu</i> ₂ ⁺		<i>psu</i> ₂ ⁺ -U11	
		1	Experiment 2 (%)	1	2 (%)
D (16)	P13	96	84	11	7
D (17)	T11	86	66	3	3
D (20)	T6	113	89	65	55
N ₁ (34)	T17	43	63	< 2	< 2
Ψ (39)	T17	87	108	103	97
T (54)	P17	68	92	13	24
Leucine tRNA					
nucleotide (position)					
D (16)	p4	112	97	95	79
D (20)	u11	64	61	53	57
N ₂ (35)	u15C	32	28	44	38
A* (38)	u15C	25	31	24	33
Ψ (40)	p12	92	85	83	93
T (65)	p15	123	87	77	91

Standard nucleotide abbreviations³ are used except that N₁ and N₂ refer to the modified residues of unknown identities that are located in the anticodons of glutamine¹ and leucine¹⁷ tRNAs, respectively. In the first column position refers to the number of residues that a nucleotide is removed from the 5' phosphate of the mature tRNA sequence. The ^{32}P -labelled precursor RNAs were isolated after infection of the ribonuclease P-deficient strain A49¹³ and were purified by polyacrylamide gel electrophoresis^{7,10}. Wild-type B/5 strain was used as the host for *psu*₂⁺ mutant 72U (Fig. 1), since this precursor RNA does not accumulate in A49 cells (our unpublished results). Modification percentages were determined by analysing the RNase A or T₁ fragment, derived from a two-dimensional fingerprint, that is indicated (fragment designations are defined elsewhere^{1,17}). The analyses involved digesting the appropriate fragment to mononucleotides with RNase T₂, fractionating the products by two-dimensional, thin-layer chromatography and then comparing the amount of ^{32}P radioactivity in modified and unmodified residues⁷. Modified residues at a level of 100% contained about 400 c.p.m.; the lower limit of detection was about 2%. Different precursor RNA preparations were used for experiments 1 and 2. In precursor RNA, glutamine and leucine tRNAs both contain the sequence GTΨCG; values for the Ψs of these two sequences were not determined because the two Ψs are in identical RNase A and RNase T₁ fragments and therefore cannot be distinguished. No evidence of modification (that is, < 2%) was observed for glutamine tRNA residue m²A (position 37) or for leucine tRNA residues D (position 17) and Gm^{2'} (position 18).

Table 1 means that all the precursor RNA molecules contain the modified residue in question. Values that fall between 2%, the lower limit of detection, and 100% reflect heterogeneity in the precursor RNA preparation with respect to a particular modified residue. Table 1 shows that the values obtained with two preparations of precursor RNA are fairly reproducible for most of the residues. We do not know if the observed fluctuations reflect genuine differences in the precursor RNA preparations or inaccuracies in the measurements. For our purpose these fluctuations are small and do not affect the validity of the major conclusions presented below.

Table 1 shows a similar set of duplicate measurements for strain *psu*₂⁺-U11. The loss of *psu*₂⁺ ochre-suppressing activity in this strain results from a C to U mutation at the 11th nucleotide from the 5' end of glutamine tRNA¹. We see that many of the modified residues are reduced in amount or are entirely absent in the *psu*₂⁺-U11 precursor RNA. Note the asymmetry in the reductions and that they are confined to the part of the precursor RNA sequence containing glutamine tRNA.

Further evidence for localisation of the effect of a mutation is provided by analysis of three additional mutants of *psu*₂⁺. Mutants *psu*₂⁺-A40 and *psu*₂⁺-U62 involve nucleotide changes in glutamine tRNA¹, whereas mutant *psu*₂⁺-72U has a C to U change at position 72 in leucine tRNA (our unpublished results). The change in notation with strain *psu*₂⁺-72U indicates that the mutant nucleotide is not in the sequence of the glutamine suppressor tRNA. Precursor cleavage yielding glutamine and leucine tRNAs is drastically reduced in mutant *psu*₂⁺-72U, which explains why this strain lacks the suppressing activity of glutamine tRNA. The top panel of Fig. 1 gives the relative positions of mutant and modified nucleotides in the glutamine-leucine precursor RNA. Figure 2 is a graphic representation of

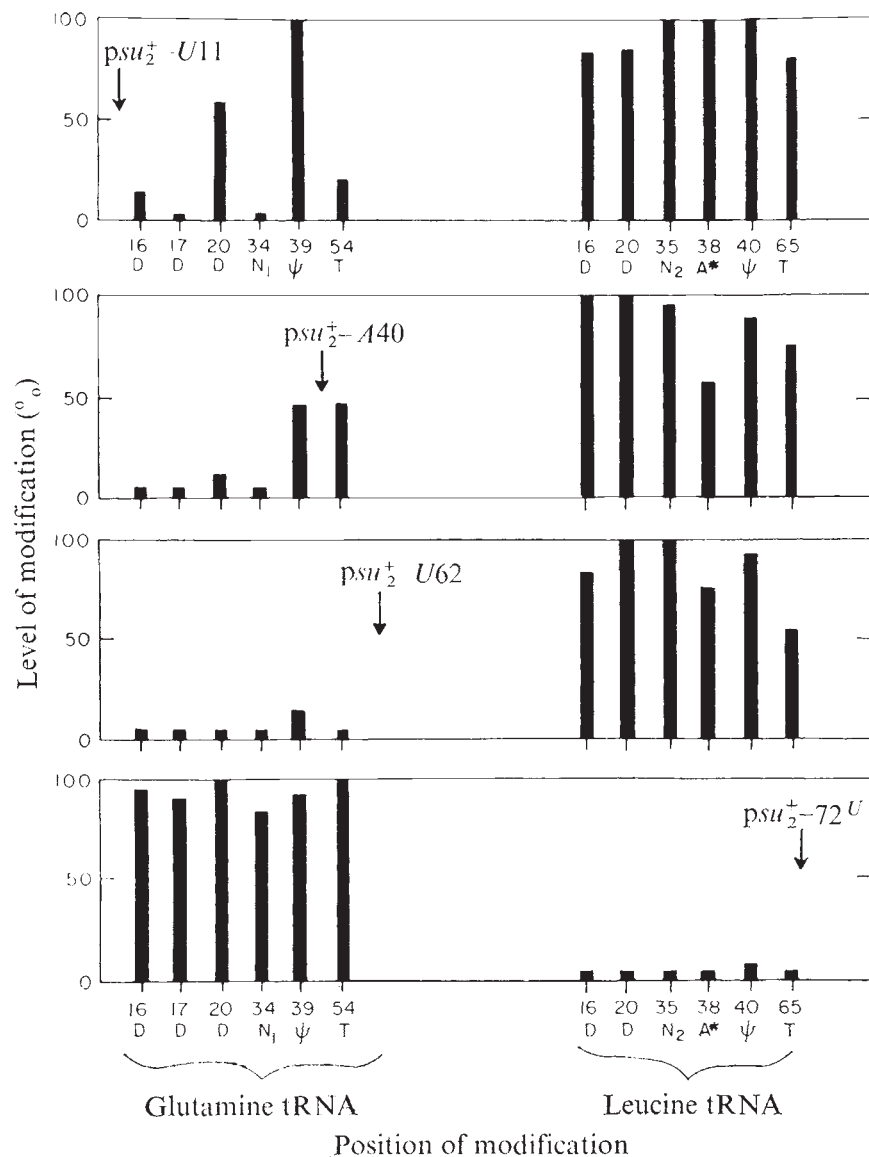


Fig. 2 Patterns of modified nucleotides in mutant glutamine-leucine precursor RNAs. The top panel displays the data in Table 1. The horizontal axis arranges the modified nucleotides with their respective residue numbers such that left to right corresponds to the 5' to 3' polarity of the RNA chain. The vertical axis gives the levels of modifications as percentages of the *psu2+* values. The arithmetic means of the duplicate determinations were used. The vertical arrow in the top panel shows where the *U11* mutation is located relative to the modified residues. The remaining panels at the bottom show analogous results obtained from single determinations with three other mutants of *psu2+*.

the modification levels in the four *psu2+* mutant precursors compared with the level of the corresponding residue in the *psu2+* precursor. The figure shows that deficiencies are confined to the tRNA sequence containing the mutant nucleotide. This is a clear demonstration of the independence of the two tRNA sequences at the time nucleotide modifications are introduced into the precursor RNA.

We obtained analogous results with the proline-serine precursor RNA that is synthesised after T4 infection^{6,7}. *psu1+* is the structural gene of an amber-suppressing serine tRNA^{8,9}. Three suppressor-negative mutants of *psu1+* involving nucleotide substitutions in serine tRNA (Fig. 1, bottom panel) were examined. Predictably, the mutant precursors were only deficient in nucleotide modifications in serine tRNA (Fig. 3).

Double cloverleaf conformation

The results in Figs 2 and 3 show that the major reductions in nucleotide modifications of precursor RNAs are confined to the tRNA sequence bearing the mutant nucleotide. There is no evidence that a mutation can have a significant effect on the formation of a modified nucleotide in the neighbouring portion of the precursor RNA containing the non-mutant tRNA sequence. These results demonstrate an independent substrate structure of the two tRNA sequences, and thus provide strong indirect evidence that the conformations of the precursor RNAs are predominated by interactions between the residues of the

individual tRNA sequences. Although the data do not specify the specific residue interactions, we suspect that they are quite similar to those found in the mature tRNAs. Such a conformation seems likely because both precursors contain only a few nucleotides in addition to those of the mature tRNAs⁵⁻⁷. We do not know the tertiary conformations of the tRNAs under consideration. The secondary structures are, however, sufficiently defined to allow for the proposal that the glutamine-leucine precursor RNA and the proline-serine precursor RNA will consist of pairs of tRNA-like cloverleaf entities as shown in Fig. 1. Although our results strictly refer to the conformations at the time of nucleotide modification, it is likely that a similar folding will characterise the molecules subsequently. Incidentally, the importance of the tertiary interactions within each cloverleaf to the substrate structure of the tRNA is documented by the absence of numerous modified nucleotides in the mutant tRNA sequences (Figs 2 and 3).

Several additional features of Figs 2 and 3 require comment. The first is the fractional modification of some residues (for example, glutamine tRNA residues D16, D20, ψ39, and T54 in mutant *psu2+* -U11). These patterns are mutant specific and presumably reflect the presence of residual precursor RNA conformations that are sufficient for modification of only a fraction of the precursor RNA chains. Such an explanation may be less likely when the same residue is fractionally modified in two or more mutants (for example, glutamine tRNA residue

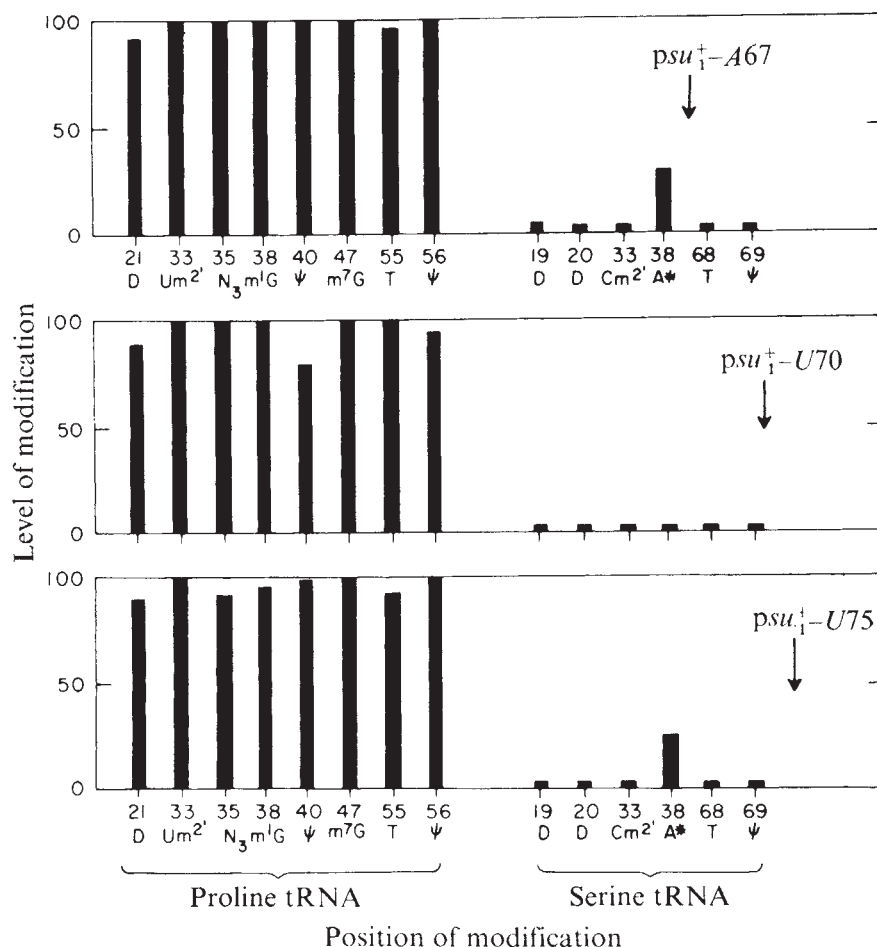


Fig. 3 Patterns of modified nucleotides in mutant proline-serine precursor RNAs. The levels of modified nucleotides are expressed as percentages of the values obtained with the *psu*₁⁺ precursor RNA. All results were obtained from single determinations. The *psu*₁⁺ values were similar to those reported previously⁷.

Ψ39 and serine tRNA residue A*38). In these cases either the recognition sites for the modifications are somewhat smaller and less specific in detail than with other residues, or there is an excess of these modification enzymes. Finally, there are indications in Figs 2 and 3 of slight reductions in nucleotide modifications in the non-mutant tRNA sequence (for example, leucine tRNA residues D16, A*38, and T65 in mutant *psu*₂⁻-U62). We do not know if these result from trivial inaccuracies in the measurements or reflect tertiary interactions between the two cloverleaf entities that influence the extent of modification. The latter possibility is potentially quite exciting, since it might offer an approach to the study of tertiary conformations of the precursor RNAs. Further experiments are in progress.

Implications for tRNA biosynthesis

Our proposal that precursor conformation closely resembles that of the mature tRNAs provides a molecular basis for understanding some of the precursor RNA biosynthetic details elaborated previously. Cleavage by ribonuclease P in the central region of the proline-serine precursor RNA is dependent on a specific nucleotide sequence at the distant 3' end of the RNA chain¹⁰. The secondary interactions of the cloverleaf bring these two distant regions of the RNA chain together, thereby enabling a single ribonuclease P molecule to interact with them as substrate for cleavage.

More generally, we suspect that a tRNA-like conformation will characterise many prokaryotic precursor RNAs. Consequently precursors of entirely different nucleotide sequences will nevertheless have similar conformations. This kind of conformational uniformity provides a common basis by which the numerous maturation enzymes can specifically recognise and handle many different precursor RNAs. The potential genetic economy that this provides is significant, since only a small amount of DNA is needed to specify an array of enzymes for maturation of many precursor RNAs. Our proposal is sup-

ported by several types of experimental results. Chang and Smith¹¹ have shown by *in vitro* chemical reactivity of the surface nucleotides that the precursor to *Escherichia coli* tyrosine tRNA has the same conformation as mature tyrosine tRNA. Unlike the phage T4 precursors described above, the tyrosine precursor contains a single tRNA species, but its maturation nevertheless involves the participation of ribonuclease P (ref. 12). Moreover, conditionally lethal mutants of *E. coli* defective in ribonuclease P fail to synthesise all tRNA species in the non-permissive conditions, and instead accumulate precursor RNAs containing one or more tRNA sequences¹³. Thus ribonuclease P is involved in the processing of precursor RNAs of diverse sizes and sequences, and it is presumably able to accomplish this by recognising the tRNA conformations in the precursor RNAs. In addition, accumulation of many different precursor RNAs in the absence of other participating maturation enzymes has also been demonstrated^{10,14,15}. Finally, the requirement of a native conformation in the precursor RNA for a productive interaction with the maturation enzymes is demonstrated by what happens when mutations like those shown in Fig. 1 destroy the conformation: they block the conversion of precursor RNA into tRNA^{1,8,9,16}. Such a safeguard for eliminating potentially deleterious tRNAs before they are functional may in part explain why precursor RNAs were implemented for the synthesis of tRNAs.

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¹ Seidman, J. G., Comer, M. M., and McClain, W. H., *J. molec. Biol.*, **90**, 677-689 (1974).

² Cedergren, R. J., and Cordeau, J. R., *J. theor. Biol.*, **39**, 477-486 (1973).

³ Nishimura, S., *Prog. nucleic Acid Res. molec. Biol.*, **12**, 49-85 (1972).

⁴ Guthrie, C., et al., *Nature new Biol.*, **246**, 6-11 (1973).

- ⁵ Guthrie, C., *J. molec. Biol.*, **95**, 529–547 (1975).
⁶ Barrell, B. G., Seidman, J. G., Guthrie, C., and McClain, W. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 413–416 (1974).
⁷ Seidman, J. G., Barrell, B. G., and McClain, W. H., *J. molec. Biol.* (in the press).
⁸ McClain, W. H., Guthrie, C., and Barrell, B. G., *J. molec. Biol.*, **81**, 157–171 (1973).
⁹ McClain, W. H., Barrell, B. G., and Seidman, J. G., *J. molec. Biol.* (in the press).
¹⁰ Seidman, J. G., and McClain, W. H., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1491–1495 (1975).
¹¹ Chang, S. E., and Smith, J. D., *Nature new Biol.*, **246**, 165–168 (1973).
¹² Robertson, H. D., Altman, S., and Smith, J. D., *J. biol. Chem.*, **247**, 5243–5251 (1972).
¹³ Schedl, P., and Primakoff, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2091–2095 (1973).
¹⁴ Seidman, J. G., Schmidt, F. J., Foss, K., and McClain, W. H., *Cell*, **5**, 389–400 (1975).
¹⁵ Sakano, H., Yamada, S., Ikemura, T., Shimura, Y., and Ozeki, H., *Nucleic Acids Res.*, **1**, 355–371 (1974).
¹⁶ Altman, S., and Smith, J. D., *Nature new Biol.*, **233**, 35–39 (1971).
¹⁷ Pinkerton, T. C., Paddock, G., and Abelson, J., *J. biol. Chem.*, **248**, 6348–6365 (1973).

letters to nature

Candidate for LMC X-5

A NEW X-ray source in the Large Magellanic Cloud, LMC X-5, has been reported¹. I wish to draw attention to the close proximity of the radio sources MC32 and MC33 to the best X-ray position. According to the positions of MC32/33 given by McGee *et al.*² they lie just outside the X-ray 90% confidence level error box for LMC X-5. In Fig. 1 I show the 6-cm radio contours of MC32/33 from ref. 2 with the X-ray error box superimposed on a plate of the region.

MC32/33 is one of six double radio sources found in the LMC by McGee and Newton³ to have one thermal and one non-thermal component. The non-thermal component (MC32) has a radio spectral index of -0.64 between 6 and 73 cm, not very different from the average value of -0.48 found by Milne⁴ for galactic supernova remnants. The association of another of these double radio sources, MC76/77, with the X-ray source LMC X-1 has previously been suggested⁵. This latter suggestion has been subsequently strengthened by X-ray observations of improved positional accuracy⁶.

Optically MC32/33 is identified with the H II region N44 of Henize⁷. Isophotes of the northern half of N44 (MC32) by

Dickel⁸ show it to have a ring-like structure which may strengthen the supernova interpretation of the object. LMC X-5 is probably a highly variable X-ray source as evidenced by the failure to detect it with Uhuru. This would imply that the X rays are emitted by a compact object (such as a neutron star) which might be expected in association with a supernova remnant.

The region around N44 is rich in early-type stars. Many X-ray sources have been identified with binary stellar systems, one component of which is a compact object and the other an early type star. One star is of particular interest—No. 73 in Sanduleak's⁹ list (indicated in Fig. 1 as Sk73) of LMC early-type stars between -68° and -69° declination. Spectroscopic observations by Feast *et al.*¹⁰ (their No. R99) indicate an emission spectrum with no visible absorption spectrum. Contamination by nearby nebulosity is an unlikely source of the emission features as the nebulosity is extremely weak in the immediate area of Sk73. I note that the He II $\gamma 4686$ line is present, a line which is frequently observed in emission in the X-ray binary stars. Photoelectric observations^{10,11} do not indicate any large amount of variability but the star may have an ultraviolet excess for its $(B-V)$ colour¹¹. Photographic photometry carried out on ADH plates (loaned by Armagh Observatory, N. Ireland) do not indicate variability in B magnitude of more than 0.15 mag.

PATRICK B. BYRNE

Dunsink Observatory,
Dublin, Ireland

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- ¹ Markert, T. H., and Clark, G. W., *Astrophys. J. Lett.*, **196**, L55 (1975).
² McGee, R. X., Brooks, J. W., and Batchelor, R. A., *Astr. J. Phys.*, **25**, 581 (1972).
³ McGee, R. X., and Newton, L. M., *Astr. J. Phys.*, **25**, 619 (1972).
⁴ Milne, D. K., *Astr. J. Phys.*, **23**, 425 (1970).
⁵ Byrne, P. B., and Butler, C. J., *Nature phys. Sci.*, **244**, 6 (1973).
⁶ Touhy, I. R., and Rapley, C. G., *Astrophys. J. Lett.*, **191**, L113 (1974).
⁷ Henize, K. G., *Astrophys. J. Suppl.*, **2**, 315, (1956).
⁸ Dickel, H. R., *Astrophys. J.*, **141**, 1306 (1965).
⁹ Sanduleak, N., *Cerro Tololo Inter-American Observatory Contrib.*, No. 89 (1970).
¹⁰ Feast, M. W., Thackeray, A. D., and Wesselink, A. J., *Mon. Not. R. astr. Soc.*, **121**, 377 (1960).
¹¹ Isserstedt, J., *Astr. Astrophys. Suppl.*, **19**, 259 (1975).

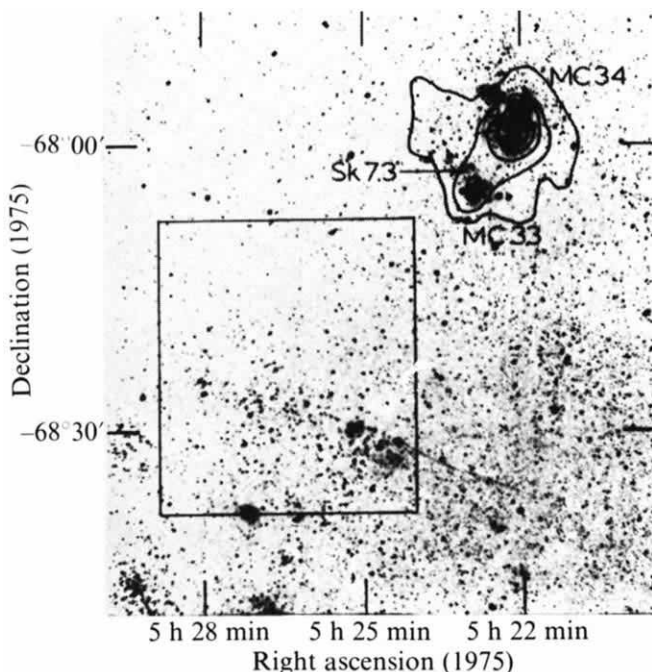


Fig. 1 The X-ray error box for LMC X-5 with the 6-cm radio contours (at intervals of 0.1 K antenna temperature) superimposed on a B plate of the region.

Internally heated convection in the atmosphere of Venus and in the laboratory

IN this letter I draw attention to the possibility that the cellular convection observed by Mariner 10 in the subsolar region of the atmosphere of Venus may be related to laboratory studies of convection with internal heat generation.

Significant regions of the Venusian convection show cellular arrays^{1,2} indicative of some form of "Bénard convection", in the general sense of convection deriving from instability of the vertical temperature gradient. The fact that the convecting region is roughly centred on the subsolar point suggests that

this unstable stratification is generated directly by solar radiation. The Venera 8 observations³ of the attenuation of sunlight with decreasing altitude show that most of the radiation absorbed by the planet is in fact absorbed in the cloud layers. On the other hand, they also show that an earlier view that it might all be absorbed very close to the cloud tops was incorrect, as is, in any case, implied by the occurrence of convection. A detailed distribution of the absorption with respect to altitude cannot be inferred from the Venera 8 observations, because of the unknown relative importance of absorption and scattering in different layers⁴. But it is highly probable that the convection is being driven by solar heating within the convecting fluid. Although cellular convection is commonly associated with the convecting layer being heated from below, it is now known to occur also in internally heated layers. Moreover, in an important respect (that is, the "elongation" of the convection cells), the Venusian convection has a closer resemblance to laboratory convection of the latter type than of the former.

The "elongation" of the convection cells means that their horizontal scale is large compared with the depth of the convecting region. The cells in the Venusian atmosphere have a horizontal size¹ (presumably to be interpreted as about twice the distance between fastest rising and fastest falling fluid) of typically 200 km; some are as large as 500 km. The top of the convecting region is thought to be at an altitude^{1,5} of 63 km. It is possible that the convection extends down to the surface of the planet, but more likely it is shallower. The Venera 8 observations³ indicate that there is no significant heat absorption below an altitude of 35 km; further analysis of these observations⁴ places the bottom of the absorbing region at any altitude between 35 km and 48.5 km. It is also possible⁶ that the temperature gradient is subadiabatic below about 45 km, although evidence on this point is contradictory³. One should further take into account the fact that the scale height of the atmosphere (about 15 km) is not large compared with the other lengths involved. The effect of this is not well understood. It will presumably tend to reduce the vertical size of the convection cells—since, in order of magnitude, one expects this size to be the smaller of the scale height and the depth of the unstably stratified region—but the modifications to the flow may be relatively minor⁶. In summary, therefore, it is apparent that, whatever method one uses to infer the vertical scale of the convection, this scale is certainly much smaller than the horizontal scale of the larger cells and probably significantly smaller than that of a typical cell. This contrasts with previous suppositions about the likely structure of convection in the Venusian atmosphere⁷.

Correspondingly, in laboratory studies of cellular convection, it was supposed that the horizontal and vertical dimensions are normally roughly equal until experiments were performed with internally heated layers⁸. Convection cells in the classical situation of a fluid layer heated at a lower boundary and cooled at an upper boundary do grow horizontally with an increasing Rayleigh number⁹, but not so much as to affect seriously the above supposition. Experiments^{8,10} with an internally heated layer, thermally insulated at the bottom and cooled at the top, give a much more dramatic growth of horizontal cell dimensions. Cells with the centres of the rising and falling regions separated by a distance of five times the layer depth have been observed, although a factor of three may be considered more typical. (A more detailed study of this situation has been made (T. Hooper and D. J. T., unpublished); some of the statements in this letter draw on those results.) Similarly elongated cells have been observed in experiments¹¹ with boundary conditions such that the convecting region lies above a stably stratified region, a case in which both horizontal and vertical cell dimensions are free to adjust.

(It should be mentioned that the interpretation of some of the experiments has given rise to some controversy¹², primarily due to the failure of theoretical work to predict the observed elongation. My reasons for believing that the observations nevertheless provide the best starting point for comparison

with natural situations will be developed elsewhere. In summary, it may be said that most of the theoretical calculations were done in a way that could not show the effect or else contained oversimplifying assumptions. A qualitatively similar, although quantitatively smaller, discrepancy between theory and experiment exists in the case of a fluid layer heated from below and cooled from above; in that case, possible causes of the disagreement on the experimental side have been much more carefully eliminated.)

It thus seems likely that, despite the small scale height and other probable complexities of the former, the basic dynamics of the Venusian convection and of the laboratory convection are similar. Quantitative comparison would imply that the larger cells in the Venusian atmosphere extend down to close to the surface, whereas the smaller ones may be vertically limited in one of the ways mentioned above. But it may well be that greater elongations than have been observed in the laboratory can occur—the dynamics of all sufficiently elongated cells being broadly similar—and thus that the convection is entirely confined to an upper atmospheric layer. Elongated cellular convection is observed in the laboratory only for a range of the Rayleigh number; we are thus supposing that small scale turbulence ("eddy viscosity" and "eddy conductivity") provides dissipative effects of the appropriate magnitude, but such a supposition is necessitated by the observation of any type of cellular convection.

Comparison of other aspects of the Venusian and laboratory convection shows general consistency. Some of the Venusian cells are polygonal, others have a more streaky appearance. In the laboratory, both polygonal and roll-like cells have been observed over different Rayleigh number ranges. The Venusian cells have highly structured interiors. In the laboratory, cells with an elongation (as defined above) of greater than about two develop an internal structure; a prominent feature is the intermittent ejection of cold blobs of fluid from the region close to the upper boundary, similar to those found in recent computational studies¹³. But these features do not sufficiently distinguish this type of convection from other types for them to be taken as supporting the main thesis of this letter.

If developing understanding of the Venusian atmosphere confirms the idea that the convection is, in its basic dynamics, similar to the laboratory convection, this may have significant implications for other situations. In particular, it may encourage application to the interior of the Earth, for which the relevance of the laboratory experiments is at present a matter of discussion¹³⁻¹⁵.

The fact that mesoscale convection in the Earth's atmosphere exhibits elongated cells¹⁶ provides a further point of comparison. The dynamical processes involved are not understood, although it is usually supposed that the elongation results from anisotropy of small scale turbulence. It is worth noting, however, that a model of mesoscale convection involving volume heat sources due to latent heat release has been developed¹⁷. The model is very different from the laboratory situation considered above—in that the heating is non-uniform and cooling due to evaporation is also involved—but it does lead to the speculation that the laboratory experiments may have closer relevance to the Earth's atmosphere also than was previously supposed⁸.

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D. J. TRITTON

*Department of Geophysics and Planetary Physics,
School of Physics, University of Newcastle upon Tyne,
Newcastle upon Tyne NE1 7RU, UK*

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- ¹ Murray, B. C., *et al.*, *Science*, **183**, 1307 (1974).
- ² JPL film 1004, *Venus Atmosphere in Motion* (Graphic Films Corp., 1974).
- ³ Marov, M. Ya., *et al.*, *Icarus*, **20**, 407 (1973).
- ⁴ Lacy, A. A., and Hansen, J. E., *Science*, **184**, 979 (1974).
- ⁵ Howard, H. T., *et al.*, *Science*, **183**, 1297 (1974).
- ⁶ Simon, G. W., and Weiss, N. O., *Z. Astrophys.*, **69**, 435 (1968).
- ⁷ Avdukevsky, V. S., *et al.*, *J. Atmos. Sci.*, **27**, 569 (1970).

- ⁸ Tritton, D. J., and Zarraga, M. N., *J. Fluid Mech.*, 30, 21 (1967).
⁹ Koschmieder, E. L., *Adv. chem. Phys.*, 26, 177 (1974).
¹⁰ de la Cruz Reyna, S., *Geofis. Int.*, 10, (2), 49 (1970).
¹¹ Kulacki, F. A., and Goldstein, R. J., *J. Fluid Mech.*, 55, 271 (1972).
¹² Schwiderski, E. W., and Schwab, H. J. A., *J. Fluid Mech.*, 48, 703 (1971).
¹³ McKenzie, D. P., Roberts, J. M., and Weiss, N. O., *J. Fluid Mech.*, 62, 465 (1974).
¹⁴ Tozer, D. C., *The Earth's Mantle*, ch. 11, (edit. by Gaskell, T. F.) (Academic, New York and London, 1967).
¹⁵ Richter, F. M., *Revs. Geophys. Space Phys.*, 11, 223 (1973).
¹⁶ Krueger, A. F., and Fritz, S., *Tellus*, 13, 1 (1961).
¹⁷ Rosmond, T. E., *J. Atmos. Sci.*, 30, 1392 (1973).

Duration of equatorial spread F

It is well established¹⁻³ that the spread-F phenomenon, which is observed as diffuse echoes on the ionograms is a common feature during night time between the dip latitudes 20°N and 20°S. Based on the study of ionograms and published monthly f_oF_2 bulletins, morphological features of spread F have been reported for several equatorial stations^{1,4-10}. Equatorial spread F as seen on the ionograms generally starts around the time of reversal of the F-region vertical motion in the evening and

continues to grow in intensity during the downward motion of the F region¹¹. Usually spread F persists only for few hours and on some occasions continues throughout the night. Sometimes, after the disappearance in the pre-midnight period, spread F reappears in the post-midnight period.

We have examined the published ionospheric data of Trivandrum (dip latitude 0.6°S, Geographic Longitude 76°52'E) for the year 1970 to study the factors influencing the duration of spread F and the results are presented in this communication. The duration of spread F is determined by using the ionograms taken at 15-min intervals. The parameter $h'F$ is taken to represent the height variations of the F region. In the year 1970 there were about 30 nights in which spread F started in the evening hours (≈ 1900 IST) and continued until the early morning. The $h'F$ variations with time have been examined for all these nights. Figure 1 shows a plot of the hourly variation of $h'F$ for ten typical long duration spread-F nights. Plotted in the same graph are the $h'F$ variations for some of the nights in which spread F was present only for 2-3 h, to compare with

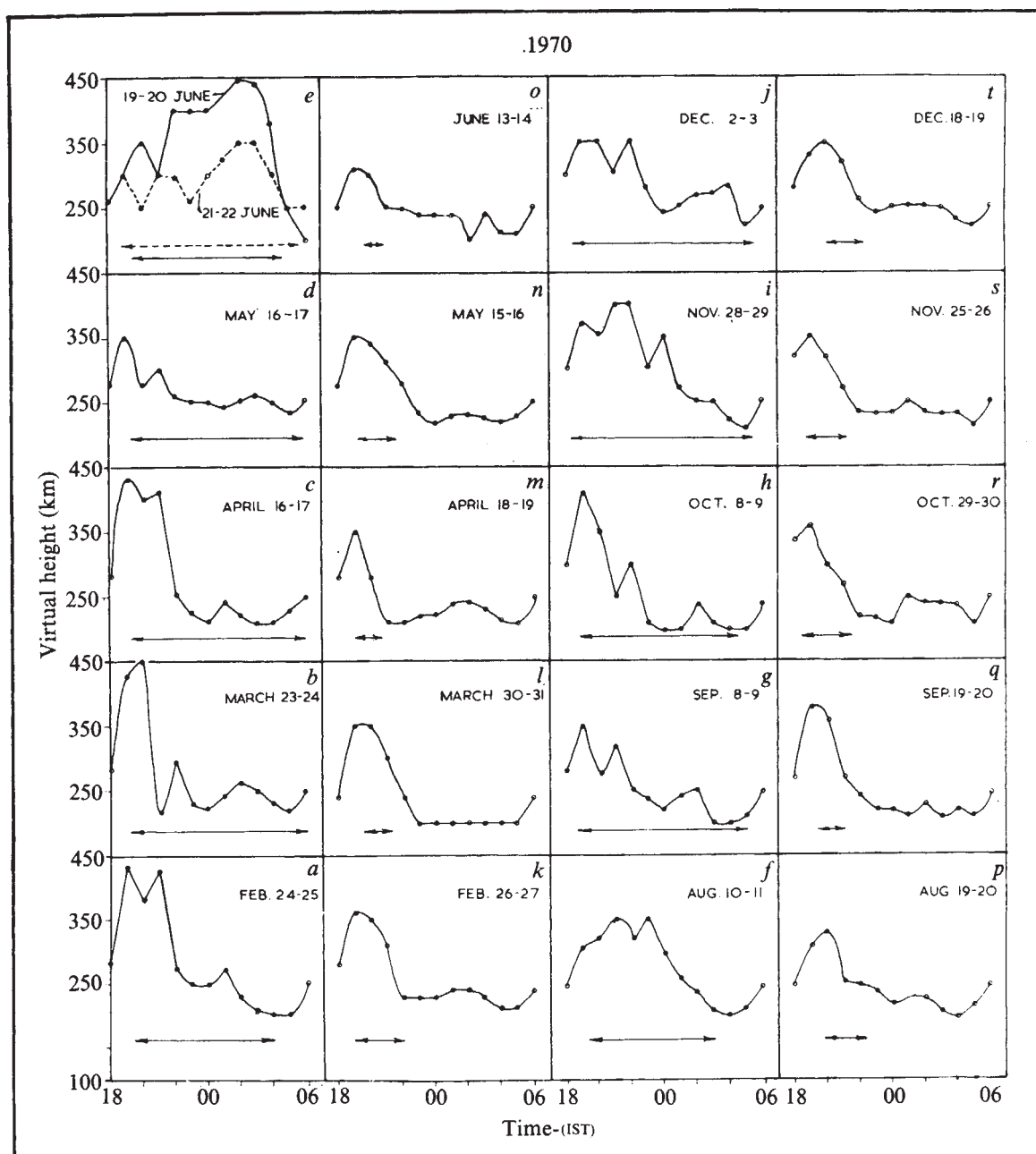


Fig. 1 $h'F$ variations during short and long duration spread-F nights. a-j, Long duration spread F; k-t, short duration spread F (2-3 h).

the $h'F$ variations during long duration spread-F nights. The duration of spread F is indicated by a line with arrows in these graphs. All the nights considered correspond to undisturbed magnetic conditions as indicated by the K_p index. Further, we have considered in this study only those nights with continuous duration of spread F. The main features of Fig. 1 are:

(1) $h'F$ starts rising from 1800 IST on all the nights shown and the onset of spread F is generally around the time of maximum $h'F$.

(2) The most striking feature is that on nights of long duration spread F, $h'F$ shows significant fluctuations. In many of these cases $h'F$ shows two peaks separated by about 1–2 h and another peak generally in the post-midnight period. On the other hand, on nights with short duration spread F, $h'F$ shows only a single maximum around 1900 IST and does not show any significant fluctuations. This is a consistent feature observed on all the nights in the year 1970 when spread F is present.

(3) On all the nights shown in Fig. 1, $h'F$ reaches approximately the same maximum height irrespective of the duration of spread F except in four cases.

(4) It is interesting that on August 10–11, 1970 (Fig. 1f) spread F continued for relatively shorter duration compared with other long duration nights and on this night, the $h'F$ fluctuations are not as prominent as they are on other nights of long duration spread F.

From an examination of the $h'F$ data on spread F nights for the whole year, it is found that even when the $h'F$ peak is above 400 km, the duration of spread F is only a few hours. Once the spread-F irregularities occur and there is no sustaining mechanism of the irregularities, these will decay in a time corresponding to the chemical loss time constant. To examine this on a quantitative basis, the chemical loss time constants are estimated corresponding to the average $h'F$ during 1900–2000, for the night of short duration spread F. For this estimation, the chemical loss coefficients given by Mitra¹² and

fluctuating east–west electric field forms one of the prerequisites of spread F generating and or sustaining mechanisms. This is consistent with the suggestion made by Hanson *et al.*¹⁴ that turbulent electric fields in the F region could be a necessary condition for equatorial spread F.

V. V. SOMAYAJULU

K. SEN GUPTA

B. V. KRISHNA MURTHY

Physics and Applied Mathematics Division,
Vikram Sarabhai Space Centre,
Trivandrum 695022, India

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- 1 Wright, R. W. H., *J. geophys. Res.*, **64**, 2203 (1959).
- 2 Lyon, A. J., Skinner, N. J., and Wright, R. W. H., *J. atmos. terr. Phys.*, **19**, 145 (1960).
- 3 Singleton, D. G., *J. geophys. Res.*, **65**, 3615 (1960).
- 4 Chandra, H., and Rastogi, R. G., *Annls Géophys.*, **28**, 37 (1972).
- 5 Booker, H. G., and Wells, H. G., *Terr. Mag. Atmos. Electr.*, **43**, 249 (1938).
- 6 Bhargava, B. N., *J. meteor. geophys.*, **9**, 35 (1958).
- 7 Chandra, H., and Rastogi, R. G., *Annls Géophys.*, **28**, 709 (1972).
- 8 Marasigan, V., *J. atmos. terr. Phys.*, **18**, 43 (1960).
- 9 Huang, Y. N., and Yeh, C. Y., *J. atmos. terr. Phys.*, **32**, 1765 (1970).
- 10 Skinner, N. J., and Kelleher, R. F., *Annls Géophys.*, **27**, 181 (1971).
- 11 Farley, D. T., Balsley, B. B., Woodman, R. F., and McClure, J. P., *J. geophys. Res.*, **75**, 7199 (1970).
- 12 Mitra, A. P., *The Chemistry of the Ionosphere* (National Physical Laboratory, New Delhi, 1970).
- 13 Martyn, D. F., *Proc. IRE*, **47**, 159 (1959).
- 14 Hanson, W. B., McClure, J. P., and Sterling, D. L., *J. geophys. Res.*, **78**, 2353 (1973).

Short term relationships between solar flares, geomagnetic storms, and tropospheric vorticity patterns

It has been found that the 500–mbar vorticity area index (VAI), which is a rough measure of the strength of the cyclonic activity over the Northern Hemisphere, responds to solar flares in at least two ways. First, there is an increase in the VAI in the first two days after the flare. Then, after the geomagnetic storm which often follows a major solar flare, there is a sharp decrease in the VAI. The results are consistent with earlier studies of the development of pressure troughs in the North Pacific area.

Many investigators have reported short term relationships between variable solar activity and weather, but the relationships have often been unconnected and even contradictory. The results reported here seem to form a logical extension of other results recently reported^{1–4}.

Roberts and Olson⁴ verified an earlier finding regarding wintertime pressure troughs at the 300 mbar level, and their apparent response to a solar-geomagnetic signal. If a day of sudden increase in geomagnetic activity is called a geomagnetic key day and the day that a trough moves into or is formed in the Gulf of Alaska is called the 0-day of the trough, they verified that the following relationship held: troughs whose 0-days fell 2–4 d after a key day were selectively intensified, on the average, during the next few days. The day of maximum intensity was about 1 d after day 0, or about 4 d after the key day.

In this and all previous work, it was very crucial that 2–4 d must elapse between the key day and day 0 for the effect to be observed. If day 0 was less than 2 d after the key day, the intensification of the trough was, on the average, missing or even negative. The geomagnetic key day was defined as any day on which the planetary geomagnetic index, A_p , equalled 15 or more, and on which the one day increase in A_p from the previous day exceeded the monthly average A_p . Thus it was defined by the sharp front of an abrupt rise in geomagnetic activity.

To measure the size and intensity of the troughs, a VAI was devised. This is defined as the area over which the absolute

Table 1 Observed and estimated spread-F data

Date (1970)	Average height (km)	Chemical loss time constant (h) (calculated)	Duration of spread F (h) (observed)
February 26–27	355	2.8	2.0
March 30–31	350	2.5	1.75
April 18–19	315	1.9	1.1
May 15–16	345	2.2	2.2
June 13–14	305	0.75	1.0
August 19–20	290	0.5	2.0
September 19–20	370	4.4	2.0
October 29–30	330	1.3	2.5
November 25–26	335	1.7	2.3
December 18–19	335	1.7	2.3

CIRA 1965 model have been used. These are shown in Table 1 along with the observed duration of spread F. There is a fairly good agreement between the observed duration of spread F and the calculated chemical loss time constant. It should be noted that the duration of spread F depends on the initial strength of the irregularities as well as on the chemical loss time constant. Considering this, the agreement between the two is quite satisfactory.

The night-time $h'F$ fluctuations represent the true height (h) fluctuations corresponding to the undersurface of the F region¹³. The variation of $h'F$ (or equivalently of the true height h) with time represents the sum of the layer velocity and the apparent upward velocity caused by the chemical loss. The latter is not expected to show the type of fluctuations in $h'F$ observed in the present investigation (Fig. 1). As such, the observed fluctuations in $h'F$ should correspond to the true vertical motion of the F region, which at the Equator is attributed mainly to the electrodynamic drift caused by an east–west electric field. From this we contend that the $h'F$ fluctuations (observed on long duration spread-F nights) indicate similar fluctuations in the east–west electric field. We suggest that the

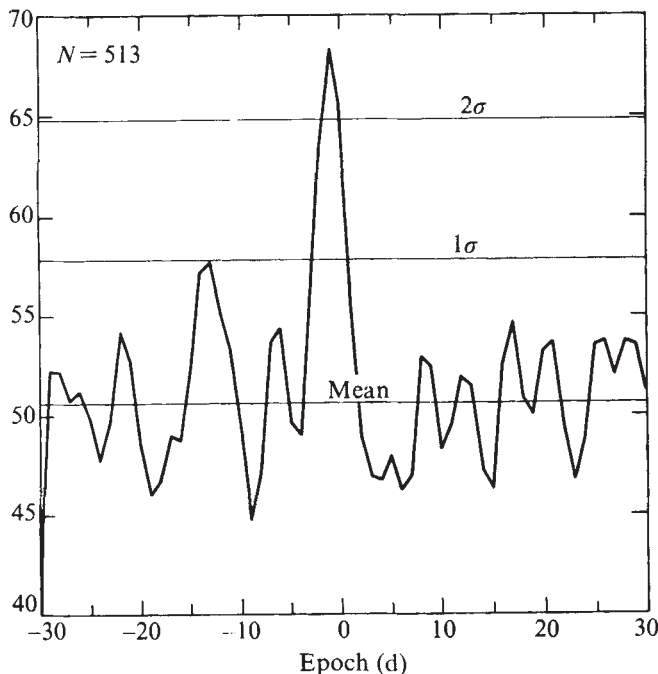


Fig. 1 Number of geomagnetic key days before and after zero days established by the three minimum value days per month of the 500 mbar VAI September–April, 1950–71.

vorticity exceeds certain threshold values. We have applied the same VAI definition to both 300 mbar and 500 mbar. We base the work in this paper on our 500-mbar results rather than those at 300 mbar, because the data at 500 mbar are more complete and homogeneous.

Wilcox *et al.*¹⁻³ have introduced a new solar signal into the study, namely the interplanetary magnetic sector boundaries that sweep past the Earth several times per month, apparently due to the Sun's rotation and the fact that they are imbedded in the solar wind and locked to magnetic field configurations on the solar surface. They also modified the VAI somewhat. Instead of matching VAI patterns with individual troughs, they integrated the VAI over the entire Northern Hemisphere north of 20°N. This makes the integrated VAI a completely objective parameter, whereas the VAI applied to individual troughs involves the degree of subjectivity necessary to identify specific troughs with vorticity maxima.

The Wilcox results, both at 500 and 300 mbar and with several independent data samples, showed a very consistent pattern. Approximately 1 d after the Earth intersects a solar sector boundary, the integrated VAI displays a 10% decrease. By 3–4 d after the crossing, the VAI has recovered to its original level or even beyond it.

Since there is a well known tendency for geomagnetic disturbances to occur near the time of sector boundary crossings, it was tempting to do a study similar to the Wilcox studies, but using geomagnetic key days as the solar signal. This report gives the results of such an experiment, plus a similar one in which major solar flare occurrence was used as the solar signal.

One of the difficulties in working with VAI data is that, although the VAI can be computed objectively by computer, the map sets needed as a basis for the calculations are somewhat non-homogeneous and therefore produce noisy data. Thus we decided in the first experiment not to use the VAI itself, but to select the three days per month when VAI was a minimum as the meteorological parameter. We then performed a superposed epoch analysis (SPE) of the number of geomagnetic key days preceding and following the three days of minimum vorticity.

The frequency of key days reaches a sharp maximum 1 d before the minimum vorticity (Fig. 1). The 1σ line on Fig. 1

was computed by the assumption of a Poisson distribution; it is the square root of the average number of key day occurrences so computed. Therefore, according to Langley⁶ the significance levels are conservative. By this estimate, the peak at day -1 is significant at almost the 1% level. Langley points out that "The Poisson answer is nearly always a shade weaker than the true binomial probability, so there is very little risk of getting a 'significant' answer when you shouldn't." It may be of interest that Langley also gives examples (ref. 6, pp. 249–250) of testing of similar distributions which give results much like ours. In addition the high value at day 0 is significant at better than the 5% level. It must be noted that not all 513 cases in the sample are independent—the days of minimum vorticity sometimes occur in clusters or even at times may be consecutive. But it is not certain that this detracts from the significance of the result. If the effect of a geomagnetic event is such that it causes several consecutive days to have low VAI values, this may support the reality of the effect.

The second part of the study involves a correlation between the occurrence and magnitude of major solar flares and the integrated VAI. Dodson and Hedeman⁵ have shown that, from a list of all well observed major flares, only the upper 20% or so in magnitude have a clear-cut causative relationship to magnetic storms. Thus in our study we used only the flares from the Dodson–Hedeman list having a combined index of 10 or more. This gave a list of 94 flares in the winter half year and 115 in the summer half, for the period 1955–1969 covered by the Dodson–Hedeman list. As a preliminary experiment we used the days of minimum vorticity, as in Fig. 1, to establish day 0, and performed an SPE analysis of flare index from day -30 to day 30.

The results (not shown here) showed only one peak, a highly significant increase in flare index at day -3. But this experiment did not add much to our knowledge, because of the well known relationship between major flares and geomagnetic disturbance. To show the relationship between solar flares and VAI in a more explicit way, we prepared Fig. 2. First, in order to remove the seasonal trend in the VAI, we subtracted each daily value from the 29-yr mean for that day. An alternative way of taking out the seasonal trend would be to subtract each daily value from a fairly extensive running mean value, for instance 27 d. We tried that technique, and again got results quite similar to those shown in Fig. 2. Even with this trend removal procedure, there is still considerable noise in the VAI data, as mentioned earlier. We performed an SPE analysis with the 0 dates determined by the occurrence of a solar flare of index 10 or more (roughly the upper 20% in flare magnitude). The VAI data appear in the form of 24-h changes, so that the data shown at the 0 point is the 24-h change in the integrated VAI from day 0 to day 1.

To test the statistical significance of the results we have plotted them in the form of Student's *t* values. The results are somewhat unexpected. In the winter half year, Fig. 2 shows a significant decrease in integrated VAI, from three to four days after the flare. This is just what we would expect, in view of Fig. 1 and the fact that these are the flares that typically produce geomagnetic storms about two to three days after the flare. But an even more significant increase in VAI occurs on the first and second day after the flare, a surprising result, since we had not seen evidence for this in other data. Although the noise level in Fig. 2 is high, we feel that both the sharp increase and the subsequent decrease in VAI are real. The increase seems significant on both days 1 and 2 following the flare. As in Fig. 1, we know of no way to incorporate the position of the peaks into a significance test. That is, if the most significant peaks and troughs occurred at day -18 or day 22, for instance, we would be hard pressed to ascribe a causative relationship to them. But since they occur near day 0, we feel that the suggestion of a physical connection must be taken seriously. The fact that a similar relationship (not shown) was found in independent data from summer months, even though it was weaker, must also be considered as evidence for the reality of the finding.

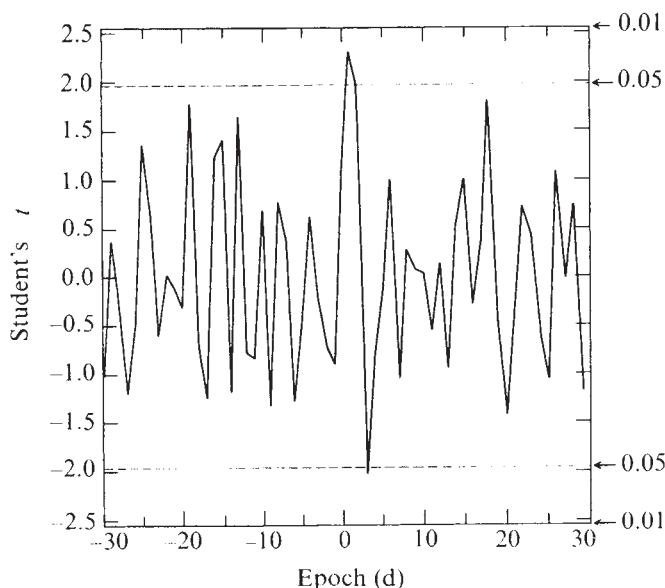


Fig. 2 Student's t values for 24-h change in Vorticity Area Index (expressed as departure from mean) before and after 94 large flares, 1955–69 (winter season, October–March). Arrows on right indicate labelled confidence limits.

From this work it is possible to suggest an average sequence of events, as follows (applicable primarily, but by no means exclusively, to the upper troposphere and to the winter half year). Starting with the day of a large solar flare as day 0:

- (1) By day 1 or 2, the Northern Hemisphere VAI increases sharply, by 5–10% above its background value;
- (2) by day 2 or 3, a geomagnetic storm has started, which may persist for several days;
- (3) by day 3 or 4, the VAI has decreased to a value 5–10% below its level before the flare;
- (4) by day 5 or 6, the VAI has recovered back to its original level.

The results of Fig. 2 can also be used to suggest a reason for the trough intensification in a large homogeneous area like the North Pacific following a geomagnetic key day. Immediately following the geomagnetic key day the integrated VAI is depressed. It is generally found that troughs in the North Pacific reflect the general hemispheric situation—they tend to be smaller when the integrated VAI is small. By 3 d after the key day, the hemispheric VAI is increasing, and the Pacific troughs apparently share in this tendency.

These results, of course, are true only in the average, and should not be taken to be case studies. Moreover several additional refinements suggest themselves. For instance, we should examine the position of the flare on the disk of the Sun as a factor in determining the VAI response. It is undoubtedly important, and the type of response obtained may give a clue as to what parameter in solar activity is most important, the ultraviolet or X-ray flux, particles, and so on. It will also be vital to examine the geographic areas of greatest response. Answers to questions like these will aid greatly in the all important problem of discovering physical mechanisms to explain the observed results. Once we have reasonably acceptable physical models, we should be enabled to perform much more critical tests of the reality of solar-weather interactions.

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R. H. OLSON

Department of Astro-Geophysics,
University of Colorado,
Boulder, Colorado 80302

W. O. ROBERTS

University Corporation for Atmospheric Research

C. S. ZEREFOS

National Center for Atmospheric Research,
PO Box 3000
Boulder, Colorado 80303

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- ¹ Wilcox, J. M., Scherrer, P. H., Svalgaard, L., Roberts, W. O., and Olson, R. H., *Science*, **180**, 185–186 (1973).
- ² Wilcox, J. M., *et al.*, *J. Atmos. Sci.*, **31**, 581–588 (1974).
- ³ Wilcox, J. M., Svalgaard, L., and Scherrer, P. H., *Nature*, **255**, 539–540 (1975).
- ⁴ Roberts, W. O., and Olson, R. H., *Rev. Geophys. Space Phys.*, **11**, 731–740 (1973).
- ⁵ Dodson, H. W., and Hedeman, E. R., *An Experimental Comprehensive Flare Index and its Derivation for 'Major' Flares, 1955–69*, Rept No. UAG-14 (NOAA, EDS, Boulder, Colorado, 1971).
- ⁶ Langley, R., *Practical Statistics Simply Explained* (Dover, New York, 1970).

Crystal structures, the Earth and Dirac's Large Numbers Hypothesis

DIRAC'S Large Numbers Hypothesis assumes that the large, dimensionless numbers obtained from the ratios between fundamental physical constants are related to the age, t , of the Universe^{1–4}. The theory demands two important consequences: that matter be continuously created with time and that the gravitational constant, G , vary with time. To reconcile those demands with Einstein's theory of gravitation two metrics are required. One, the Einstein metric, involves the equations of motion and classical mechanics; the other, the atomic metric, refers to quantum mechanics and laboratory measurements of distances and time as determined by atoms. According to Dirac, the continuous creation of mass requires either of two models—one, called additive creation, assumes that matter is created uniformly throughout space (mostly intergalactic space). The other model is called multiplicative creation. It calls for the creation of matter where matter already exists in proportion to the amount and kind already existing.

A corollary of the multiplicative creation model is that Einstein's cylindrical closed Universe suit cosmological considerations. Adopting the Einstein metric, and using the Einstein field equations, Dirac argues that if the radius of the Universe and G were constant and the galaxies were not receding then all atomic distances, expressed in Einstein units, would vary proportionally to t^{-1} . In terms of Einstein units, the number of atomic particles varies as t^2 whereas the mass of atomic particles varies as t^{-2} . In that way the mass of a classical body can remain constant. The fundamental atomic constants of physics all vary in a compensatory fashion, so, presumably, as 'new' atoms are added to 'old' atoms all atoms are changing so that at any point in time 'old' and 'new' atoms are indistinguishable.

Suggested tests of the theory³ remain unconfirmed. Measurements of astronomical distances with atomic apparatus could provide a test as could highly accurate, secular measurements of G . The Earth can also provide tests for the theory. Adopting the atomic metric, mass and atomic distances remain constant, but as G varies the Earth would expand and move away from the Sun^{1,5,6}. Continental drift and plate tectonics are, in part, consistent with this test of the atomic metric.

Crystals in rocks of the Earth represent a record of possible change from at least 3×10^9 years ago up to the present so their atomic structures should agree with the predictions of the theory. When dealing with atomic units no problem emerges as mass and atomic distance remain constant so no differences between old and new crystals can be expected since any variation in G would be negligible compared with the atomic forces involved. But when dealing with Einstein units major changes are demanded at the atomic level. Dirac² has stated that "There might be some difficulty in understanding how the matter in very old rocks can have multiplied without disrupting their crystal structure". If atomic distances vary as t^{-1} then the d -spacings in crystal lattices in geologically older minerals should

be observably different from those in the same minerals forming today. The geological evidence, however, is strongly against such a prediction: the lattice dimensions of a 3×10^9 yr-old quartz crystal are the same as the lattice dimensions of quartz grown in the laboratory. Indeed, the molecular weight and the X-ray density of the unit cells are the same. Therefore, unless Avogadro's hypothesis is violated, the number of atoms in the unit cell is also the same. The relevant equation from X-ray crystallography is:

$$V = Mn/pN_0 \quad (1)$$

where, respectively, V is the lattice volume, n is the number of atoms, M is the molecular weight, p is the X-ray density of the crystal unit cell, and N_0 is Avogadro's number. The equation must be valid in both atomic units and Einstein units. But no difference in any of these parameters is observable between an early Precambrian crystal and a modern crystal of the same composition. Therefore, in Einstein units, this condition violates the predictions of the multiplicative creation model.

Gittus⁸ has offered an apparent solution to this difficulty and has suggested that the 'new' atoms could occupy interstitial positions at vacancies and dislocations. Calculations show that for a crystal 3×10^9 yr old, if $n \propto t^2$ more than 30% of the atoms would have to occupy interstitial positions. Although this is clearly too many for any crystal to accept without lattice disruption it would, in any case, have to produce a marked difference between the X-ray and pycnometrically determined densities.

The geological evidence does not confirm the predictions of the multiplicative creation model and unless Avogadro's number is to be held constant with time, similar difficulties arise for the additive creation model. In its present form, therefore, the Large Numbers Hypothesis seems to be unacceptable.

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KENNETH M. TOWE

Department of Paleobiology,
Smithsonian Institution,
Washington, DC 20560

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- ¹ Dirac, P. A. M., in *The Physicist's Conception of Nature* (edit. by Mehra, J.), 45 (Reidel, Dordrecht, 1973).
- ² Dirac, P. A. M., *Naturwissenschaften*, **60**, 529 (1973).
- ³ Dirac, P. A. M., *Proc. R. Soc., A338*, 439 (1974).
- ⁴ Dirac, P. A. M., *Nature*, **139**, 323 (1937).
- ⁵ Dicke, R. H., *Science*, **138**, 653 (1962).
- ⁶ Hoyle, F., *Q. Jl. R. astr. Soc.*, **13**, 328 (1972).
- ⁷ Gittus, J. H., *Proc. R. Soc., A343*, 155 (1975).

Origin of alkaline rocks

THE hypothesis of limestone assimilation^{1,2}, developed to explain differentiation of the Vesuvius magma, was long unchallenged for that area—and extended³—even when it had to be abandoned for other alkaline provinces. Trace element data made it difficult to reconcile the limestone assimilation hypothesis with many observations^{4,7}, but even in 1966 when Hurley *et al.*⁵ challenged Rittmann's hypothesis¹ (on the basis of Sr isotope data) they had insufficient evidence to rule it out. But Pb isotope data support⁸ the genetic model of Hurley *et al.*⁵, and further evidence against Rittmann's hypothesis is presented here.

The main difficulty Hurley *et al.* encountered results from the overlap of the $^{87}\text{Sr}/^{86}\text{Sr}$ values found in limestone and in the alkaline rocks from Vesuvius and the other volcanic areas they investigated. The Sr isotopic composition of limestone is determined by two components: The Sr in the carbonate will have the isotopic composition of seawater at the time of deposition, assuming no later Sr exchange. Clay minerals in the limestone will generally have retained their inherited isotopic composition. Peterman *et al.*⁹ found a range in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for seawater of 0.707–0.709 with the least radiogenic Sr

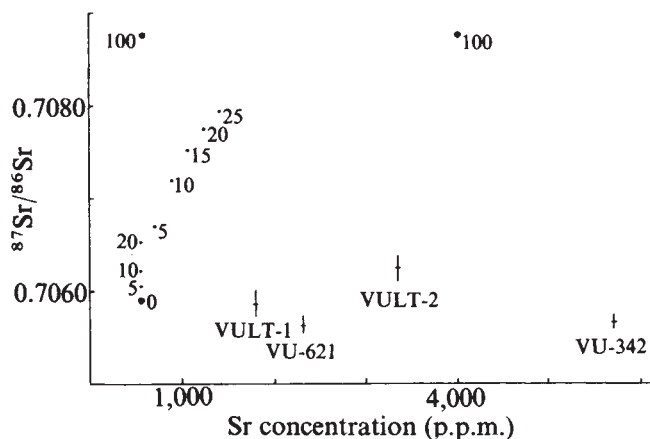


Fig. 1 The change in the Sr isotopic composition which would be produced by the assimilation of limestone with 610 p.p.m. Sr (average limestone¹⁴) or evaporites with 4,000 p.p.m. Sr (possible, though rather high value¹⁵) is plotted as percentage of assimilated sediment. The magma is assumed to have 550 p.p.m. Sr and an isotopic composition similar to the rocks from Vulture. The Sr isotopic composition of the sediments is assumed to be equal to Miocene seawater⁹. The data from Vulture are taken from ref. 8.

in the Jurassic and Cretaceous and a rapid increase in the ratio during the Tertiary. These will be minimum values. Impure limestone tends to have higher values due to inherited radiogenic Sr in the clay minerals. For example, average values of 0.7129 and 0.7141 for Jurassic and Cretaceous limestones were obtained¹⁰ as compared with 0.707 for pure limestone of the same ages. Impure limestone will have less radiogenic Sr than pure limestone only in the rare cases when a significant amount of the total Sr originates from volcanic material with non-radiogenic Sr derived from the mantle. Thus, an attempt to refute the limestone assimilation hypothesis will be facilitated, if the uncontaminated magma has a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio below the minimum value for seawater. New Sr isotope determinations on the Italian alkaline rocks⁸ have shown that this seems to be the case for the Cainozoic¹¹ volcanic complex of Mt Vulture in Southern Italy. The assimilation of limestone¹² or Miocene evaporites¹³ has been suggested as an explanation for the occurrence of haüyne and leucite in rocks from Mt Vulture.

Assuming Sr concentrations for the uncontaminated magma and the sediment, the effect on the isotopic composition resulting from these hypotheses is demonstrated in Fig. 1. None of the predicted correlations can be observed. The range in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is very small and the mugearite (VULT-2), which according to the hypothesis should have suffered little or no contamination, has in fact a ratio which is slightly higher than the rest of the samples. Furthermore, the haüynofyr (VU-342), which according to the hypothesis should have the highest portion of assimilated sediments, has the lowest isotope ratio, which is in contradiction to the hypothesis. Thus, a characteristic feature of these rocks—the very high Sr concentrations—cannot have been caused by the assimilation of a sediment.

As more Sr isotope data on the Italian alkaline rocks become known, a pattern emerges, which enables one to test Rittmann's hypothesis also at Vesuvius. The Sr isotope ratio is rather constant throughout the eruption sequence for each volcanic centre (with a total range generally less than 0.0012) and each centre has a distinct range. This indicates individual and quite uniform sources. The Somma–Vesuvius volcano seems to be the one exception. Here relatively large variations in $^{87}\text{Sr}/^{86}\text{Sr}$ which ranges from 0.7068 to 0.7100, are found. Even within the same lava flow almost the same spread can be observed: two samples from the 1944 lava flow yield 0.7072 (ref. 8) and 0.7095 (ref. 10). These exceptional large variations and the inhomogeneities within a single lava flow point to a superficial contamination with Sr. This might well be caused by the abundant

limestone inclusions which are subjected to various degrees of metamorphic alteration¹. But if Rittmann's hypothesis were correct, that increasing assimilation with time of dolomite and limestone would have produced the successive desilification of the Vesuvian lavas, the Sr isotopic composition would be expected to correlate with the eruption sequence and the silica content. Neither can be observed.

This line of argument only breaks down if the exceptionally large variations are not caused by superficial contamination, or the contamination has a different origin, for which no evidence has yet been put forward. Then any Sr contribution from the limestone inclusions would be masked and the Sr tracer method could not be used to detect any possible limestone assimilation at Vesuvius.

R. VOLLMER

Laboratory for Isotope Geochemistry and Mass Spectrometry,
Swiss Federal Institute of Technology, Zurich, Switzerland

Received June 23; accepted July 14, 1975.

Present address: Department of Earth Sciences, University of Leeds, UK.

- ¹ Rittman, A., *Z. Vulkanol.*, **15**, 8 (1933).
- ² Daly, R. A., *Bull. geol. Soc. Am.*, **21**, 81 (1910).
- ³ Burri, C., *Rend. Soc. min. Ital.*, **17**, 3 (1961).
- ⁴ Holmes, A., and Harwood, H. F., *Q. Jl geol. Soc.*, **88**, 370 (1932).
- ⁵ Hurley, P. M., Fairbairn, H. W., and Pinson, W. H., *Earth planet. Sci. Lett.*, **5**, 301 (1966).
- ⁶ Savelli, C., *Contrib. Min. Petrol.*, **16**, 328 (1967); *ibid.*, **18**, 43 (1968).
- ⁷ Capaldi, G., Gasparini, P., Moauro, A., Salvia, E., and Travaglion, O., *Earth planet. Sci. Lett.*, **17**, 247 (1972).
- ⁸ Vollmer, R., *Geochim. cosmochim. Acta* (in the press).
- ⁹ Peterman, Z. E., Hedge, C. E., and Tourtelot, H. A., *Geochim. cosmochim. Acta*, **34**, 105 (1970).
- ¹⁰ Hoefs, J., and Wedepohl, K. H., *Contrib. min. Petrol.*, **19**, 328 (1968).
- ¹¹ Cortini, M., *Riv. Ital. Geol.* (in the press).
- ¹² Burri, C., *Rend. Accad. Sc. fis. mat. della Soc. Naz. Sci., Lett. Arti, Napoli*, **28**, 131 (1961).
- ¹³ Hieke-Merlin, O., *Mem. Ist. Geol. Min. Univ. Padova*, **26** (1967).
- ¹⁴ Turekian, K. K., and Kulp, J. L., *Geochim. cosmochim. Acta*, **10**, 245 (1956).
- ¹⁵ Braitsch, O., *Salt deposits, their origin and composition* (Springer, Berlin, 1971).

Laboratory-induced self reversal of thermoremanent magnetisation in pillow basalts

CONSIDERABLE attention has been paid to the changes in the magnetic properties of pillow lavas with distance from a ridge¹. It has been found^{2,3} that the originally stoichiometric titanomagnetites are altered at sea-floor temperatures to cation-deficient titanomagnetites (titanomaghaemites). The degree of cation deficiency—the fraction of initial Fe^{2+} oxidised to Fe^{3+} —is described by the parameter 'z' (ref. 4). As the titanomaghaemite evolves, all the magnetic properties (for example, Curie point, saturation magnetisation, coercive force) change, but though the changes cause a decrease in the intensity of magnetisation, the direction is preserved.

We have attempted to duplicate the process of oxidation in the laboratory. In our experiments thin disks (2.5 cm in diameter, by 1.5 mm thick) of basalt were cut from cores drilled into the pillows. Each slice was progressively demagnetised in an alternating field up to a peak of 1,000 oersted. Next, the sample was kept in a field 1.0 of gauss at 150 °C in water at typical sea-floor pressures (~0.5 kbar) for a length of time, then cooled to room temperature in the 1.0 gauss field, and finally the thermoremanent magnetisation (TRM) was demagnetised in an alternating field (a.f.). This cycle was repeated with increasing time intervals until over 3,000 h at 150 °C had been accumulated. Two experiments were carried out on pillows dredged from the Median Valley of the Mid-Atlantic Ridge at 45 °N.

The radial variation of the composition of the titanomagnetites was measured³. By taking the lowest Curie temperature phase as stoichiometric titanomagnetite an x -value of 0.6 is obtained. If it is assumed that x remains constant throughout, then z can be obtained from the Curie temperature.

The a.f. demagnetisation curves for the partial thermal remanences (PTRMs) acquired by the first sample (56-3-35—the 35 indicating a sample 35 mm from the pillow rim), which had

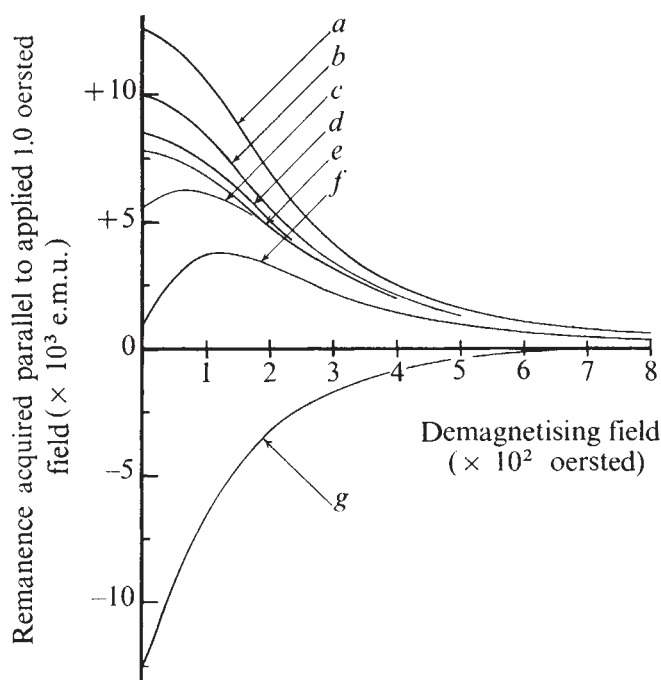


Fig. 1 Alternating field demagnetisation curves for successive PTRMs acquired by sample 56-3-35 on cooling from 150 °C in a 1.0-oersted field. Cumulative times at 150 °C: a, 46 h; b, 174 h; c, 1,070 h; d, 3,545 h; e, 588 h; f, 3,370 h; g, 3,110 h.

an initial $z \approx 0.35$, are shown in Fig. 1. First there was a long interval during which PTRM acquisition slowly decreased with a corresponding slight increase in hardness, seen by normalising the curves. Next the sample started to acquire a component of remanence anti parallel to the external field, until after 3,110 h it had reversed completely. After further heating the PTRM reverted to normal and became indistinguishable from PTRM acquired at much shorter times. This sort of transient self reversal is similar to that described by Ozima and Ozima⁵.

In the second experiment one sample showed complete self reversal, two showed very small reverse components and two showed no reversal at all. The a.f. demagnetisations of the PTRMs for the self-reversed sample (197-8-45), with initial value of $z \approx 0.15$, are shown in Fig. 2. The final curve in this case clearly represents an intermediate state between the 1,070 and 3,110 h states for sample 56-3-35. Curie point measurements at successive stages of alteration for this sample showed an increase in 'z' to about 0.7 followed by splitting of the cation-deficient titanomagnetite so that a higher Curie point (~450 °C) phase is identifiable as well as a phase with the initial Curie point. Self reversal can be shown to accompany the phase splitting in some samples. An attempt to speed the reaction by heating at 210 °C caused phase splitting but no self reversal.

We suggest that the self reversal is caused by an interaction between the titanium-poor daughter phase with the Curie point at 450 °C and the original titanomagnetite. Domains of daughter phase will acquire a chemical remanent magnetisation (CRM) parallel to the external field as they exsolve from the parent. This will occur as they grow in size during the time spent at 150 °C, increasing their relaxation time, until they are normally magnetised, stable, single domains⁶. On cooling, the previously a.f. demagnetised, mother phase will be subjected to both the external field and an interaction field from the daughter phase with which it is in contact. It is conceivable that the interaction field will dominate and thus the mother phase will acquire a reverse PTRM. It is possible that the reverse PTRM of the mother phase will cause a net reverse moment in the sample^{6,7}.

It is not possible to decide definitely whether this is a magneto-static or an exchange interaction. Experiments with stronger

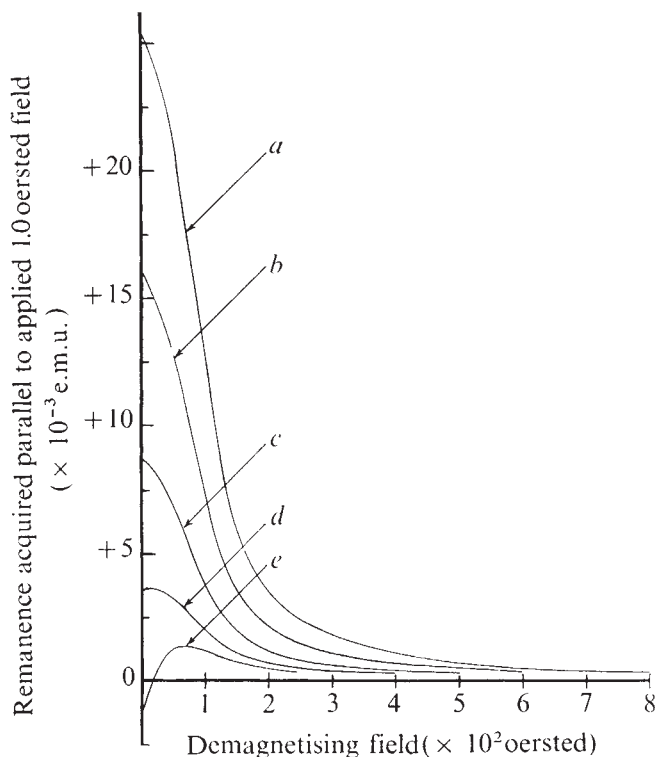


Fig. 2 Alternating field demagnetisation curves for successive PTRMs acquired by sample 197-8-45 on cooling from 150 °C in a 1.0-oersted field. Cumulative times at 150 °C: a, 82 h; b, 166 h; c, 622 h; d, 2,351 h; e, 3,023 h.

fields may be able to resolve this question. The reverse PTRMs are, however, softer (mean demagnetisation field = 80 oersted; compare with 200 oersted; MDF = 80 oersted; compare with 140 oersted for the two samples) than the normal PTRMs. It has been found^{8,9} that the reverse TRM caused by exchange interaction is harder than the normal TRM, so perhaps the present case is an example of magnetostatic interaction.

An important question is whether we should anticipate self-reversed basalts in the ocean crust. In nature the daughter regions could acquire a reverse CRM as they are exsolved in the interaction field of the mother regions. A necessary condition for self reversal is the splitting of the original titanomagnetite into two or more phases. During the phase splitting, conditions exist which may lead to self reversal. Phase splitting of titanomagnetites caused by oxidation on the sea floor at very low temperatures has not been observed. It is, however, well known from laboratory¹⁰ and field studies¹¹ that alteration at 100 °C to 150 °C causes phase splitting in basaltic titanomagnetites.

These experiments suggest that though self-reversed rocks may not be found on the sea floor, they may occur wherever the temperature has been sufficiently raised to split the cation-deficient titanomagnetite into two or more phases, perhaps at depth in the oceanic crust and perhaps in continental regions of hydrothermal alteration.

PATRICK J. C. RYALL
J. M. ADE-HALL

Department of Geology,
Dalhousie University, Halifax,
Nova Scotia, Canada

Received May 7; accepted July 7, 1975.

- ¹ Irving, E., *Can. J. Earth Sci.*, **7**, 1528-1538 (1970).
- ² Marshall, M., and Cox, A., *J. geophys. Res.*, **77**, 6459-6469 (1972).
- ³ Ryall, P. J. C., and Ade-Hall, J. M., *Can. J. Earth Sci.*, (in the press).
- ⁴ O'Reilly, W., and Banerjee, S. K., *Nature*, **211**, 26-28 (1966).
- ⁵ Ozima, M., and Ozima, M., *Earth planet. Sci. Lett.*, **3**, 213-215 (1967).
- ⁶ Néel, L., *Adv. Phys.*, **4**, 191-243 (1955).

- ⁷ Graham, J. W., *J. geophys. Res.*, **58**, 243-260 (1953).
- ⁸ Uyeda, S., *Jap. J. Geophys.*, **2**, 1-123 (1958).
- ⁹ Ozima, M., and Larson, E. E., *J. Geomagn. Geoelect. Kyoto*, **20**, 337-351 (1968).
- ¹⁰ Johnson, H. P., and Merrill, R. T., *J. geophys. Res.*, **78**, 4938-4949 (1973).
- ¹¹ Ade-Hall, J. M., Palmer, H. C., and Hubbard, T. P., *Geophys. J.*, **24**, 137-174 (1971).

Sliding friction of rubber

WE report experiments demonstrating that it is possible to predict quantitatively the level of friction arising when a smooth rubber sphere slides on glass. Analysis of the sliding friction is based on a surface energy approach. Observations show that when a smooth rubber surface advances over glass by a continuous peel process (Schallamach waves) the work done in the region of contact can be calculated in terms of a rate-dependent surface energy and thus an expression found for the tangential frictional stress required to maintain uniform relative motion between the surfaces. In some cases predicted and observed frictional stresses agree to within 10%.

Schallamach observed¹ that for sliding of soft rubber spheres on hard tracks relative motion between them was often only due to "waves of detachment" crossing the region

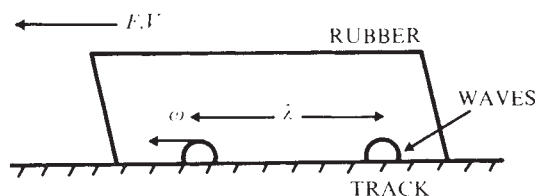


Fig. 1 Generation of Schallamach waves at rubber-track interface. $F = \gamma\omega/\lambda V$.

of contact. Between these waves there was strong adhesion. The waves moved as folds in the rubber surface and all gross displacement seemed to be solely associated with them. More recent observations² have revealed some conditions for their appearance. The waves have been thought to originate from elastic^{1,3} or viscoelastic² instability due to tangential stresses in the contact region, so causing the rubber surface to buckle. Before the rubber surface can buckle to form a wave, however, it must first be unpeeled from the hard track¹. To unpeel smooth soft rubber from glass requires considerable energy^{4,5} and the process is greatly influenced by the viscoelastic behaviour of the rubber^{6,7}. There is evidence that the apparent surface energy required to peel apart these surfaces is of the form $\gamma = \gamma_0 f$ (hysteresis) where γ_0 is the equilibrium energy associated

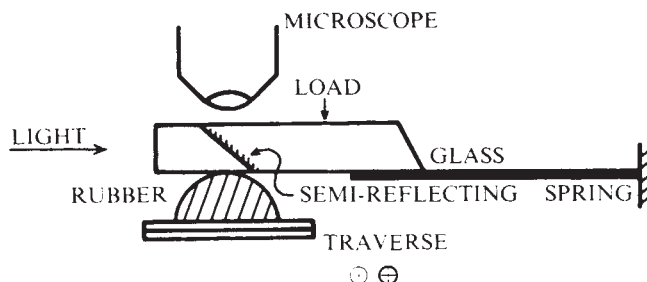


Fig. 2 Apparatus to investigate the role of Schallamach waves in sliding rubber friction. The glass plate is a prism beamsplitter designed for viewing the contact region between rubber and glass in high optical contrast⁸. Rubber hemispheres of various vulcanisates were moulded to give an optically smooth surface¹⁰ of radius 1.85 cm. A variable speed electric motor drove a traverse to move a hemisphere back and forth ($\odot \pm$ perpendicular to plane of paper) under the glass plate.

Table 1 Wave observations and the friction of rubber hemispheres on glass

Rubber vulcanisate (Hardness IRHD)	Body velocity V ($\mu\text{m s}^{-1}$)	Centre of contact observations			Frictional stress (MPa)		
		ω (mm s^{-1})	λ (mm)	γ (J m^{-2})	Theory Central	Theory Integrated	Observed
Natural rubber							
peroxide cure (35)	590	17.4 ± 4	1.3 ± 0.2	5.98	0.14	0.16 ± 0.03	0.15 ± 0.02
sulphur cure (44)	240	5.9 ± 0.6	0.34 ± 0.1	5.01	0.36	0.53 ± 0.015	0.48 ± 0.04
Polychloroprene (48)	100	2.3 ± 0.5	0.62 ± 0.1	21.4	0.79	0.83 ± 0.07	0.70 ± 0.03
Styrenebutadiene (50)	110	2.9 ± 0.3	0.52 ± 0.08	9.12	0.46	0.44 ± 0.02	0.45 ± 0.03
Butyl rubber (34)	50	0.59 ± 0.06	1.2 ± 0.2	15.1	0.15	0.16 ± 0.03	0.20 ± 0.03
Polybutadiene (53)	110	4.0 ± 0.8	0.34 ± 0.08	1.78	0.19	0.20 ± 0.03	0.26 ± 0.03
Acrylonitrilebutadiene (43)	30	0.70 ± 0.2	0.6 ± 0.1	5.76	0.22	0.22 ± 0.06	0.29 ± 0.02

with the change in interfacial area and where the function of hysteresis may be up to 10^4 in magnitude⁸. Thus the question arose as to whether the unpeeling of rubber from the track was the main origin of the frictional work that is done in sliding the sample⁴.

The idea of a rate-dependent surface energy has been applied to Schallamach waves in the following way⁴. Consider a rubber slab pulled at a velocity V over a hard track by a tangential stress F (Fig. 1). The waves move with a velocity ω and their spacing apart is λ . If γ is the rate-dependent surface energy per unit area required to peel the rubber from the track and if the energy dissipation in the bulk of the rubber is neglected, then in steady-state sliding where the elastic energy of the rubber is not varying the loss of energy is solely associated with the

peeling. In time dt the stress F does work $FVdt$ per unit area and the energy lost by waves is $(\gamma\omega/\lambda)dt$. Equating these gives

$$F = \gamma\omega/\lambda V \quad (1)$$

Some energy is returned each time surfaces readhere in the wake of a Schallamach wave, but this is negligible¹ compared to that lost in peeling. The validity of equation (1) was assessed approximately by using the published wave observations of others^{1,2} together with estimates of γ at the relevant rates of peeling ω and it has been shown⁴ to predict the correct order of magnitude for the sliding frictional force of a smooth rubber contact.

To investigate more directly the relation between surface energy and rubber sliding friction we have constructed apparatus to measure simultaneously frictional stress, wave velocity and wave spacing for the sliding at uniform velocity of a smooth spherical rubber surface over a polished glass plate (Fig. 2). The plate normally loaded on the rubber was attached to a supporting spring, tangential deflections of which gave a measure of the frictional force between the sliding surfaces. The sliding contact area was viewed through a low power microscope and by using a double light flash technique it was possible from a single photograph to deduce the wave velocity ω and spacing λ (Fig. 3). Any wave passing through the contact was photographed twice and from the displacement, knowing the time interval between flashes, its velocity ω could be found. Measurement of the distance between waves of the same flash set gives λ . The surface energy γ is rate dependent and was determined from independent sets of experiments; measurement of the contact radius between a smooth sphere of rubber and a glass plate with no applied load yielded a value for the surface energy required to separate the surfaces¹¹, the rate at which the surfaces were separated determined the magnitude of γ for a particular vulcanisate⁴. Thus for a range of vulcanisates rate-dependent γ values were found. At the peel rates set by the Schallamach waves, γ values are as much as two or three orders of magnitude greater than the equilibrium surface energy γ_0 generally found for hydrocarbon surfaces.

The experimental observations are shown in Table 1. If average values of ω and λ are taken midway across the contact, agreement between theory and experiment is good. The Schallamach waves, however, do not travel at a uniform velocity². From photographs such as Fig. 3, it was possible to measure accurately the variation in velocity ω and spacing λ of the waves as they crossed the contact area. Point values of the frictional stress F were calculated using equation (1) and then summed across the contact to arrive at a more accurate theoretical value of the total frictional stress. In some cases the integrated theoretical frictional stress is within 10% of the direct observations (Table 1).

Thus it seems that for a wide range of vulcanisates, at least in the case of smooth surfaces, surface energy of peeling probably has a central role in the frictional behaviour of rubber. Furthermore, this picture gives a ready mechanism for the known viscoelastic behaviour of rubber friction because the peeling process has already been established as being viscoelastic in character. This is believed to be the first time such an



Fig. 3 Typical double flash photograph of Schallamach waves for natural rubber. The duration of each flash was $1/1,200$ s and the interval between flashes could be varied between $1/100$ s up to 1 s using a simple delay system. To distinguish clearly between each set of waves one light flash was arranged to be slightly less intense than the other so that on a black and white photograph one wave set appears darker than the other. The major axis diameter of the elliptical contact area in this photograph was 3.2 mm at a sliding velocity of $570 \mu\text{m s}^{-1}$ under a normal load of 0.085 kg.

approach has been taken to provide a quantitative description of rubber friction based on independently measurable physical quantities. Such a picture avoids having to invoke any specific structure or molecular jump theory.

A. D. ROBERTS
S. A. JACKSON

Malaysian Rubber Producers' Research Association,
Brickendonbury, Hertford SG13 8NP, UK

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- ¹ Schallamach, A., *Wear*, **17**, 301-312 (1971).
- ² Barquins, M., and Courtel, R., *Wear*, **32**, 133-150 (1975).
- ³ Gent, A. N., *Wear*, **29**, 111-116 (1974).
- ⁴ Roberts, A. D., and Thomas, A. G., *Wear*, **33**, 45-64 (1975).
- ⁵ Kendall, K., *J. Polym. Sci. A2*, **12**, 295-301 (1974).
- ⁶ Gent, A. N., and Petrich, R. P., *Proc. R. Soc.*, **A310**, 433-448 (1969).
- ⁷ Andrews, E. H., and Kinloch, A. J., *J. Polym. Sci. A2*, **11**, 269-273 (1973).
- ⁸ Gent, A. N., and Schultz, J., *Proc. Int. Rubber Conf.*, paper C1 (IRI, London, 1972).
- ⁹ Roberts, A. D., *J. Phys. D.*, **4**, 423-432 (1971).
- ¹⁰ Roberts, A. D., *Eng. Mater. Des.*, **11**, 579-580 (1968).
- ¹¹ Johnson, K. L., Kendall, K., and Roberts, A. D., *Proc. R. Soc.*, **A324**, 301-313 (1971).

Relaxation of the Bernal model

RANDOM packings of spheres serve as models for a variety of noncrystalline particulate and molecular aggregates, some of which, for example the amorphous phases of metals and alloys, offer abundant possibilities for the comparison of theory and experiment¹⁻⁴. Nevertheless there remain significant discrepancies between the measured structural characteristics of real systems and the geometrical models considered by Bernal⁵, Cohen and Turnbull⁶ and others to be prototypes for the simple vitreous state. The most enduring of these is perhaps the difference in position and intensity between the two components of the split second peak in the radial distribution function observed in actual and artificial systems²⁻⁴. Attempts to remove this disagreement by variation of the algorithm used in computer-simulated packings have only been partially successful. In particular Sadoc *et al.*² have noted that it is relatively easy to build in strong local density inhomogeneities associated with icosahedral arrangements⁷⁻⁸ and Connell has experimented with the packing of compressible spheres, showing that significant shifts in the radial distribution function result from the softening of the interatomic potential⁹.

We thought it worthwhile to 'relax' the '1968' (ref. 10) Bernal sphere-packing model under a realistic interatomic potential function to observe which of the resultant geometrical changes can occur fairly easily in an initially dense packing, as distinct

Table 1 Frequency of polyhedron types

	Hard sphere	Relaxed	Crystal close to melting point†
'Crystalline'			
(0 3 6)*	0.32	0.34	0.40
(0 4 4)	0.22	0.27	0.44
(0 5 2)	0.06	0.03	0.07
	0.60	0.64	0.91
'Non-crystalline'			
(0 0 12)	0.02	0.04	<0.01
(0 1 10)	0.08	0.11	0.01
(0 2 8)	0.14	0.17	0.06
	0.24	0.32	0.07

Only clearly significant types are shown. Hence the proportions given do not total to unity.

*The polyhedron type ($n_3 n_4 n_5$) is characterised by n_3 , n_4 , n_5 faces with three, four and five edges, respectively.

†This column refers to a Monte Carlo simulation of crystalline argon at 84 K using a Lennard-Jones potential function¹⁰.

from those which may need to be induced in the building process. We used quite standard computational techniques for the 'optimisation' of many-variable potential functions¹¹, applying a Lennard-Jones 6:12 potential between all atoms present and allowing the program to seek out a natural potential energy minimum in the neighbourhood of the initial 'Bernal' configuration. Even with the best programs available, however, we could not have hoped to handle the whole 7,934-atom Bernal packing and so were obliged to extract a central core of some 999 atoms for the calculation.

The algorithm used was based on a version of the Fletcher-Reeves conjugate-gradient method¹², supplied to us by the Theoretical Physics Division, AERE, Harwell, and run on the London University CDC 7600 machine. We took precautions to see that the program searched thoroughly in the near vicinity of the original point in configuration space, avoiding as far as possible the unnatural rearrangement of atomic positions which might have occurred with a 'dynamic' optimisation method. Nevertheless, the final configuration obtained cannot be expected to be in any way unique and there will undoubtedly be a multitude of alternative minima at similar or smaller distances, from the 'Bernal' point on our 2,991-dimensional energy surface. We assume only that the short-range geometrical correlations at the minimum found shall be statistically representative of the whole class of alternatives which might be discovered by repeated excursions in the neighbourhood of the 'Bernal' configuration.

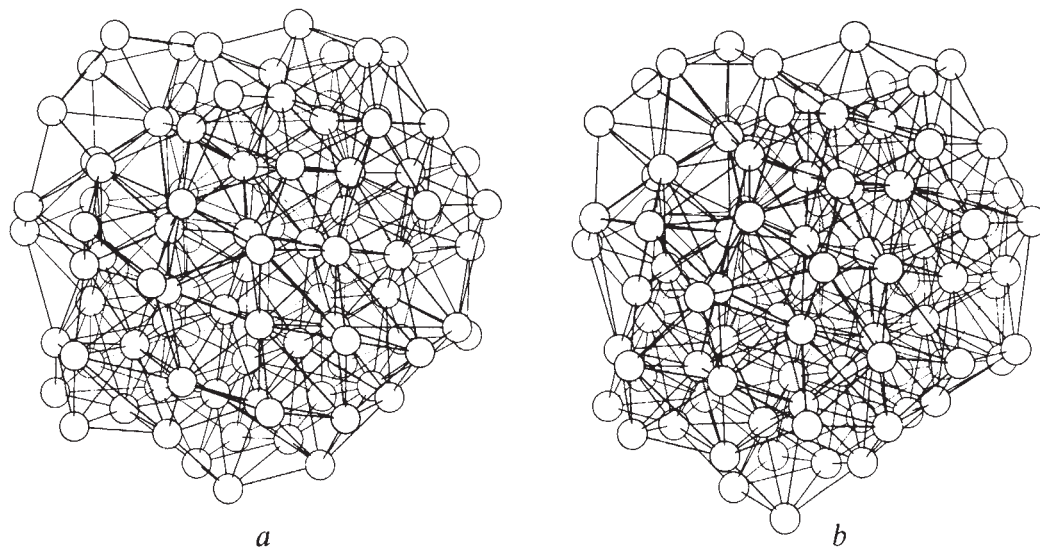


Fig. 1 The Bernal model before (a) and after (b) relaxation under the Lennard-Jones potential. Only the 100-atom core of the 999-atom assembly is depicted. Atoms within a distance $\sqrt{2}$ of each other are shown bonded. The radius of the spheres is drawn at 0.15 of the diatomic equilibrium distance.

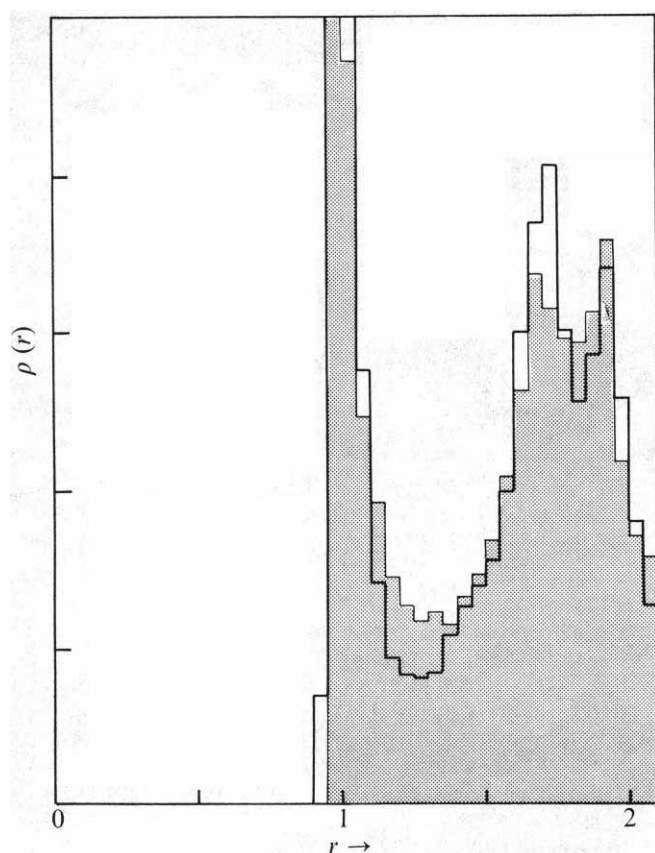


Fig. 2 Radial distribution function for the relaxed and unrelaxed Bernal model. The histogram for the original system (shaded) is superimposed on that for the relaxed (blank). Distance in terms of the Lennard-Jones pair distance. Vertical scale in arbitrary units.

The results of this exercise can be presented in various ways. Fig. 1 shows computer-generated drawings of the central core of 100 atoms extracted from the 999-atom aggregate before and after relaxation. We were able to scrutinise many such drawings at different angles and in stereoscopic form to confirm the impression given by the figure that perceptible changes have taken place in the spatial relationship of neighbouring atoms even though, for the most part, the topology of nearest-neighbour connections is relatively undisturbed.

The resulting structural changes in fact gave rise to a corresponding decrease in total potential energy of some 5.1% from the Bernal configuration. This, we estimate, would have decreased further by some 0.3% had we continued computing for quite unjustified times. The r.m.s. distance moved by the spheres was also monitored and amounted to approximately one-fifth of the nearest-neighbour distance averaged over the whole assembly. Detailed examination of the coordinates showed that a disproportionate contribution to this figure came from atoms at or near the surface of the model which underwent relatively large adjustments.

Figure 2 shows the averaged radial distribution function of the 999-atom model before and after relaxation, computed using the Mason correction¹³. As expected, the first peak becomes less sharp while the first minimum is deepened. More significantly, the two components of the split second peak reverse in intensity in precisely the manner observed in amorphous Ni-P and other disordered alloys²⁻⁴. There is also a suggestion of a splitting in the smaller third peak. Otherwise the radial distribution function is unexceptional and the peak positions, which in general can be expected to depend somewhat on the nature of the potential, remain virtually unchanged for the Lennard-Jones case.

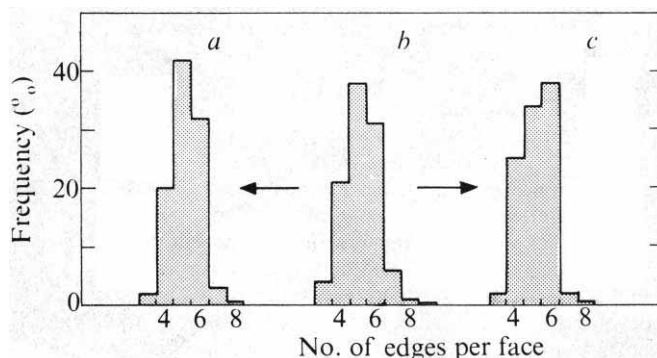
A more sensitive indication of the changes in local structure is to be found in the statistics of the Voronoi polyhedra¹⁵ before and after relaxation. Table 1 shows the frequency of some particularly significant polyhedron types for the random packing before and after relaxation, in comparison with those for the crystal near the melting point. There are significant changes in the statistics of faces per polyhedron which together lead to a reduction in the average number of faces $\bar{N}$ from 14.20 in the Bernal configuration to some 13.99 in the relaxed form. (Compare values of 14.46 and 14.07 for Lennard-Jones liquid and solid phases, respectively, at the melting point¹⁰.) On more detailed analysis we observed the following:

- (1) The size distribution of normalised polyhedron volumes $V_i/\bar{V}$ indicating the variation of local volumes effectively occupied by atoms shows relatively slight adjustment on relaxation. A slight downward shift in the maximum is observed with only small change in standard deviation (0.039 and 0.037, respectively, before and after). This indicates that the overall volume decrease in the packing goes with a redistribution of local volumes rather than simply a rescaling.
- (2) The frequency of the five-edge faces increases at the expense of others, particularly the three- and seven-face components, but otherwise the qualitative difference between 'random' and 'crystalline' polyhedron statistics is maintained (Fig. 3).
- (3) On analysing the statistics of occurrence of actual polyhedron types rather than of faces, significant trends may be distinguished. Thus the occurrence of the (0 0 12) polyhedron, indicative of distorted icosahedral arrangements, shows an appreciable increase in the relaxed array (Table 1). This trend is also shared by the (0 1 10) and (0 2 8) types which also arise from the distorted icosahedron. Crystallisation must result in a large increase in the occurrence of (0 4 4) and (0 3 6) types; these contribute little in the original packing and show no particular increase in relaxation.

The most striking single feature of these results is undoubtedly the fall in the average face-count $\bar{N}$ on relaxation. In view of (1) to (3) and the lack of any visual evidence in the drawings, this cannot be attributed to incipient crystallisation and we conclude that we have observed a spontaneous reinforcement of locally 'tetrahedral' order^{9,11}, perhaps occasioned by the formation of distorted motifs related to the 13-atom icosahedron. That these do not seem to have occurred on the scale predicted in the experiments of Sadoc *et al.* is not surprising in as much as we have taken as our initial configuration the particularly dense arrangement based on the Bernal ball-bearing model. Even with the soft potential used, this may have introduced unnatural constraints, with atoms 'jammed' and unable to take up more efficient minimum configurations without passing by way of complicated high-dimensional saddle points that are inaccessible with the program used.

How worthwhile it may be to elaborate further packing structures without reference to the physical deposition process

Fig. 3 Comparison of the Voronoi polyhedron face-frequency for: a, the relaxed Bernal model; b, the hard-sphere Bernal model; c, the Lennard-Jones crystal at melting point.



it is desired to model seems debatable. There may simply not be a single canonical structure appropriate to vitreous packings generated in unspecified experimental conditions. In any case it seems necessary to assert that, in view of the above results, the Bernal-Cohen-Turnbull view of the random close-packed structure as one essentially determined by hard-sphere repulsions and a particular, limited set of basic polyhedral relationships may need refinement when applied to the aggregation of real atoms under definite thermodynamic and mechanical constraints.

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J. A. BARKER
M. R. HOARE

Department of Physics,
Bedford College,
Regent's Park, London NW1 4NS, UK

J. L. FINNEY

Department of Crystallography,
Birkbeck College,
Malet Street, London WC1 7HX, UK

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- ¹ Cargill, G. S., *J. appl. Phys.*, **41**, 12–29 (1970).
- ² Sadoc, J. F., Dixmier, J., and Guinier, A., *J. Noncryst. Solids*, **12**, 46–60 (1973).
- ³ Finney, J. L., *J. Phys. Colloq.*, **36**, C2 1–11 (1975).
- ⁴ Leung, P. K., and Wright, J. G., *Phil. Mag.*, **30**, 185–194 (1974); **30**, 995–1008 (1974).
- ⁵ Bernal, J. D., *Proc. R. Soc.*, **A280**, 299–322 (1964).
- ⁶ Cohen, M. H., and Turnbull, D., *Nature*, **203**, 964 (1964).
- ⁷ Frank, F. C., *Proc. R. Soc.*, **A215**, 43–46 (1952).
- ⁸ Frank, F. C., and Kasper, J. S., *Acta Crystallogr.*, **11**, 184–190 (1958).
- ⁹ Connell, G. A. N., *Solid State Commun.*, **16**, 109–112 (1975).
- ¹⁰ Finney, J. L., *Proc. R. Soc.*, **A319**, 495–507 (1970).
- ¹¹ Hoare, M. R., and Pal, P., *J. Cryst. Growth*, **17**, 77–96 (1972).
- ¹² Fletcher, R., and Reeves, C. M., *Computer J.*, **7**, 149–154 (1964).
- ¹³ Mason, G., *Nature*, **217**, 733–735 (1968).

Examination of ancient pottery using the scanning electron microscope

A SEQUENCE of pottery sherds from Iraq spanning the period about 6000 BC to 750 AD and two sherds from Turkey about 5000 BC have been examined using a scanning electron microscope (SEM). This examination provided information on the internal morphology developed during firing and in particular information on the extent of vitrification (the glassy phase) and the pore structure^{1,2}. The extent of vitrification provided a useful parameter for characterising the quality of the pottery since it influences several physical properties (such as, hardness, strength, permeability) which are relevant to its suitability for the various uses to which it might be put. In addition by refiring samples of the pottery at known temperatures and determining, by re-examination with the SEM, the temperature at which an increase in the vitrification had occurred, it was possible to estimate the firing temperature used in antiquity.

Fresh fracture surfaces in the pottery, coated with a thin layer of Au-Pd, were examined in a SEM (Cambridge S600) both before and after refiring at known temperatures in the range 750 to 1,250 °C. The refirings were carried out in a tubular furnace in air at a heating rate of 200 °C h⁻¹ with a soaking time of 1 h at the peak temperature.

The nature of the internal morphology observed with the SEM together with X-ray powder diffraction data established that all the pottery was manufactured from calcareous clays. On the basis of the stage which had been reached in the progressive development of vitrification, it was possible to divide the pottery into four groups (Table 1). The first group (NV) contains pottery in which there is no vitrification and the

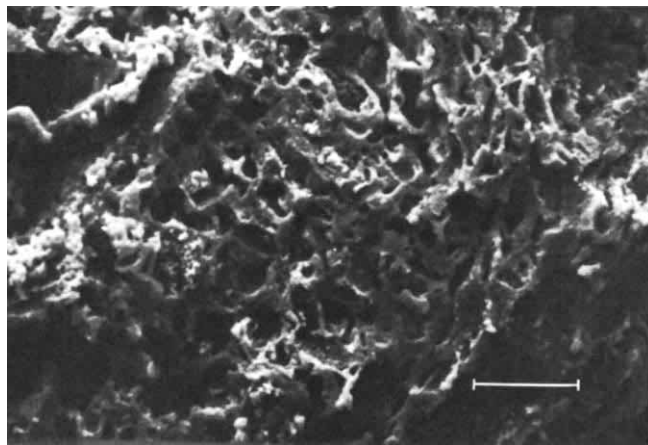


Fig. 1 Scanning electron micrograph of extensively vitrified pottery sherd (V) from Nineveh (Neo-Assyrian), Iraq. Bar represents 10 µm.

aggregates of flaky clay particles are essentially the same as in raw clay. The second group (V) exhibits extensive vitrification with a network of smooth-surfaced glass filaments forming an open or cellular structure over the fracture surface (Fig. 1). In the third group (V+), the cellular structure has begun to coarsen and larger areas of glass are present. Finally the fourth group (TV) exhibits total vitrification, the cellular structure having disappeared and been replaced by a continuous vitrified surface containing isolated pores (Fig. 2).

By means of refiring experiments on the pottery, together with the data obtained from examining specimens of calcareous clays fired at known temperatures and showing a similar pattern of vitrification development, it was possible to assign approximate firing temperature ranges to the various vitrification stages as indicated in Table 1. In assigning these ranges, it was assumed that the firing times (heating plus soaking time) employed in manufacture were comparable with those employed during refiring. Significantly faster heating rates (say 800 °C h⁻¹) and shorter soaking times (5 min) would have required firing temperatures higher by about 50 °C to achieve the same extent of vitrification. Second, on the basis of the colour of the pottery, it was assumed that it had been fired in an oxidising atmosphere. If fired in a reducing atmosphere, firing temperatures lower by about 50 °C would have produced an equivalent vitrification.

The pottery from Iraq was all fired to sufficiently high temperatures to produce, at least, extensive vitrification (V)

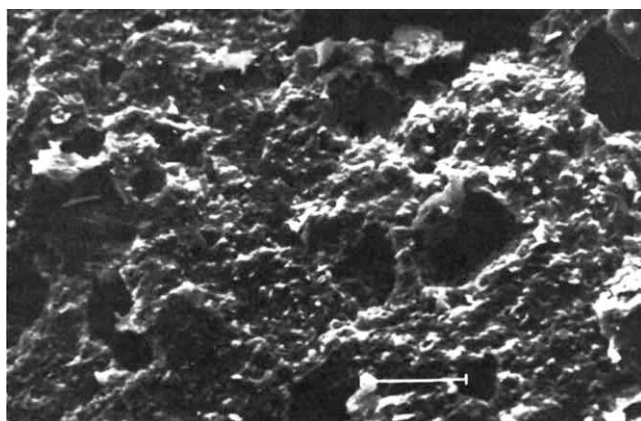


Fig. 2 Scanning electron micrograph of totally vitrified pottery sherd (TV) from Ubaid, Iraq. Bar represents 10 µm.

Table 1 Vitrification stage and firing temperature of ancient pottery

Provenance*: Period	Approximate time ranges	Number of sherds			
		NV < 800 °C	V 850–1050 °C	V+ 1050–1150 °C	TV > 1150 °C
Choga Mami (Jarmo)	c. 6000 BC		1		
Samarra	c. 5500–5000 BC		2	1	
Arpachiyah (Halaf)	c. 5000–4500 BC		4		
Ubaid, Ur, Eridu (Ubaid)	c. 4500–4000 BC		1	2	4
Nineveh: IV/V	c. 3500–2800 BC		3	1	1
Jemdet Nasr	c. 3500–3000 BC		1	1	
Kish: Early Dynastic	c. 2900–2400 BC		3		
Kish: Old Babylonian	c. 1800 BC		1		
Nineveh: Neo-Assyrian	c. 800–600 BC		2		
Nineveh, Kish: Parthian	c. 150 BC–250 AD		3		
Kish: Sasanian	c. 250–650 AD		1		
Hacilar	c. 5000 BC	2			

*Type-site given in brackets where this differs from provenance. Except for Hacilar, which is in Turkey, all the sites are in Iraq.

and for most of the periods under consideration, this group predominates (Table 1). The principal exception is the Ubaid pottery which exhibits chiefly total vitrification (V+ and TV), indicating that consistently higher firing temperatures were employed during this period. It is therefore apparent that the technology required to manufacture extensively vitrified pottery using calcareous clays was developed in Iraq at a very early stage in the production of pottery and that this tradition in ceramic technology remained essentially unchanged over a 6,000-yr period. Furthermore the tradition in Iraq contrasts with that in Turkey during the period around 5000 BC where, although calcareous clays were again used, the firing temperatures employed were significantly lower and the pottery produced exhibits no vitrification (NV).

The use of calcareous clays, although obviously determined in part by their availability, is of interest since this type of clay possesses distinctive vitrification properties which facilitate firing². First, extensive vitrification (V) can be achieved at the comparatively low firing temperature of about 850 °C. Second, the characteristic cellular structure associated with extensive vitrification remains essentially unchanged over a temperature range of about 200 °C (from 850 to 1,050 °C) and therefore the control of the temperature attained during firing is not particularly critical. This contrasts with the situation for non-calcareous clays where the extent of vitrification increases progressively with increasing temperature so that the quality of the pottery produced can vary quite considerably with changes in firing temperature of about 50 °C. But in the context of estimating the firing temperatures employed in antiquity, the thermally stable structure associated with calcareous clays results in a wide temperature range being assigned to the extensive vitrification group (V) in Table 1.

These results establish that, because information is obtained on both the extent of vitrification and the firing temperature, the examination of ancient pottery with an SEM is valuable for characterising and distinguishing between the different traditions in ceramic technology in antiquity. Since the firing temperature required to produce a particular degree of vitrification can vary by as much as 200 °C, depending on the refractoriness of the clay and the firing atmosphere used, either the extent of vitrification or the firing temperature by itself does not fully characterise the ceramic technology. For example, the same extent of vitrification can be achieved by firing a calcareous clay at a low temperature (850 °C) or a refractory non-calcareous clay at a higher temperature (950–1,000 °C). Taken together, however, the extent of vitrification and the firing temperature reflect the technological capabilities of the ancient potters with respect to both their ability to achieve the necessary temperatures and their understanding of the refractory properties of the clay used. In this respect, therefore, the examination of ancient pottery with an SEM is more valuable than an estimate of the firing temperature by itself,

such as can be obtained either by studying those minerals altered during firing^{3,4} or by thermal expansion measurements⁵.

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M. S. TITE
Y. MANIATIS

Department of Physics,
University of Essex, Colchester, UK

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- Freeman, I. L., and Rayment, D. L., *Trans. Brit. Ceram. Soc.*, **67**, 611–618 (1968).
- Tite, M. S., and Maniatis, Y., *Trans. Brit. Ceram. Soc.*, **74**, 19–22 (1975).
- Perinet, G., *Trans. Seventh Intern. Ceramic Congress*, 371–376 (1960).
- Cousins, D. R., and Dharmawardena, K. G., *Nature*, **223**, 732–733 (1969).
- Tite, M. S., *Nature*, **222**, 81 (1969).

Temperature-independent relaxation in a lamellar block copolymer

It has long been assumed that all processes giving rise to stress relaxation in high polymers are very dependent on temperature. The peak in mechanical or dielectric loss factor ($\tan\delta$) can be used to characterise the most probable relaxation time of a high polymer solid. The temperature locus of this relaxation time is described by a simple Arrhenius-type law for low temperature, glassy state processes¹ and by the Williams, Landel and Ferry expression² for ' T_g ' processes. We now report results for an elastomer exhibiting a unique relaxation process which remains essentially constant in position and magnitude over a temperature range of more than 100 K.

The block copolymer used was a poly(α -methylstyrene-*b*-dimethylsiloxane) prepared with 'star' geometry $(AB)_4X$, where A represents a poly(α -methylstyrene) block, B a poly(dimethylsiloxane) block and X is a tetrafunctional coupling atom. This polymer was synthesised by Dr D. Jones, Dow Corning Company, by sequential anionic copolymerisation³. The product contained 37.5% (w/w) poly(α -methylstyrene), molecular weight 9,000, with the molecular weight of the B block 15,000. In common with other block copolymers, different morphologies can be achieved by differing the physical treatment⁴. In the present case lamellar morphology could be generated by compression moulding a sheet (523 K, 13 MPa (130 bar)) with considerable flow in the plane of the sheet or by slow casting from benzene on a mercury surface. Dynamic mechanical measurements were carried out either in bending geometry (bars $\approx 20 \times 10 \times 2$ mm) to give Young's modulus (E') or in shear sandwich geometry (cubes with side 2 mm) to give the rigidity over wide ranges of temperature and frequency on a slightly modified version of a phase- and amplitude-measuring mechanical spectrometer previously reported^{5,6}.

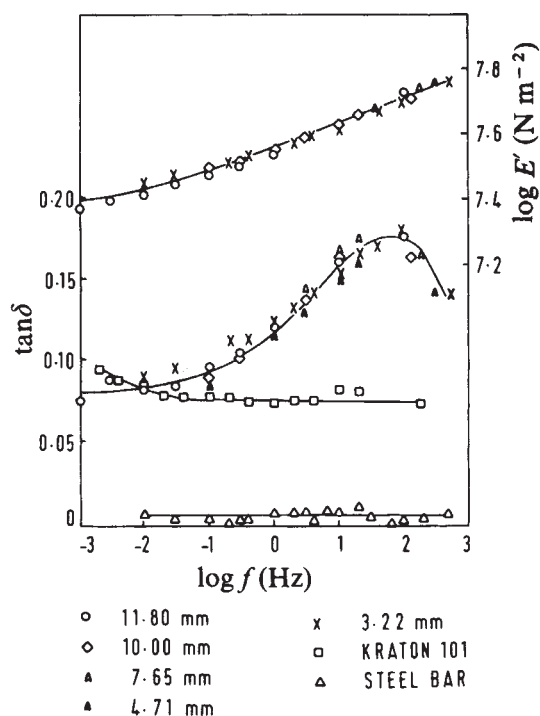


Fig. 1 Frequency dependence of mechanical loss factor ($\tan\delta$) and Young's modulus (E') for a benzene-cast sample (294 K) in the bending mode with different clamped lengths as indicated.

Results are shown in Fig. 1 for the benzene-cast sample in a bending mode perpendicular to the sheet direction at 294 K. A clear mechanical loss peak occurs at about 90 Hz and the data remain constant with temperature as shown in Fig. 2. Proper working of the apparatus was checked using standard samples (a high tensile steel bar and a Kraton 101 (Shell) block copolymer) with the results reported in Fig. 1; no abnormalities were evident. Further checks were made by measuring five bars of different lengths of the copolymer under study. The data all lie on the same curve, indicating the reality of the result and the accuracy of the measurements.

Both methods used to produce the lamellar morphology also cause orientation of the lamellae in the plane of the cast or moulded sheet. Small angle X-ray diffraction has been used to characterise both the lamellar spacing and orientation, using both slit and pinhole optics as described previously⁷. Four orders of diffraction are observed, with the ratio of the derived d spacings 1:0.52:0.335:0.254 (from pinhole optics and photographic detection) for the moulded sample and 1:0.489:0.328:0.242 for the benzene-cast sample giving certain evidence of lamellar structure. Pinhole data were used to establish that uniaxial symmetry exists about the normal to the plane of the sheet and to evaluate the orientation function⁸ $\langle \cos^2\phi \rangle$ where ϕ is the angle between lamellar planes and the perpendicular to the sheet. The orientation function is 0 for perfect orientation and 0.333 for random orientation. The benzene-cast sample gave $\langle \cos^2\phi \rangle = 0.215$ and the more highly orientated, moulded sample $\langle \cos^2\phi \rangle = 0.016$.

The mechanical properties of these oriented systems will clearly be anisotropic and their directional dependence has been studied for the experimentally accessible situations, which will be reported by us in full elsewhere. Cubes were cut from the sheet of the benzene-cast sample and clamped so that shearing occurred on the faces of the sheet (geometry 1) and perpendicular to the sheet (geometry 2). The relaxation processes are shown for the pertinent samples in Fig. 3. The simplest case of shearing in the plane of the lamellae (geometry 1) produces a low modulus (10 MN m^{-2}) and also a low magnitude transition, whereas

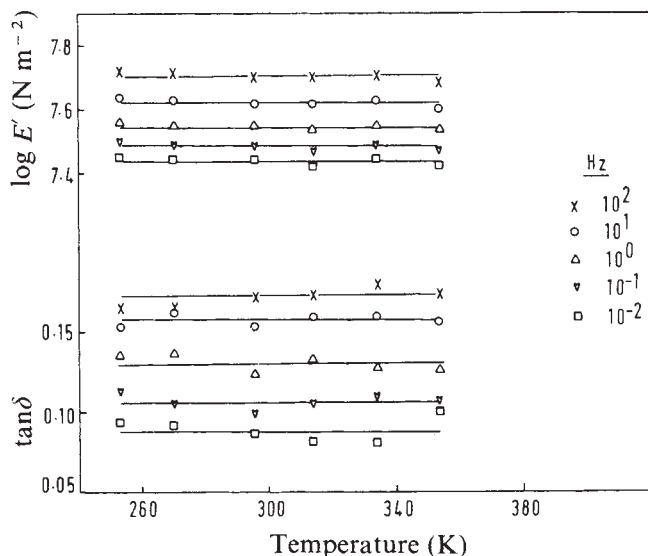
shear perpendicular to their plane (geometry 2) produces a larger loss peak. This indicates that shearing processes alone between lamellae are not responsible for the mechanical loss. In the Young's modulus measurements, the highly oriented moulded sample exhibits a low relaxation magnitude whereas the corresponding benzene-cast sample with inferior orientation yields a large relaxation magnitude, as does shear with geometry 2. This is good evidence that bending of lamellae is a key feature of the loss process, with lamellae at large angles to the stress direction deforming by bending before undergoing possible rotation and shear.

The results do not conform to the simple interlamellar shear model of Iwayanagi⁹ nor to the more refined model of McCrum and Morris¹⁰, both of which were proposed for semi-crystalline polymers. Two models are now proposed for the relaxation processes: (1) cantilever bending with relaxation by interlamellar shear and rotation, and (2) cantilever bending in a viscous medium. Each is a 'pack of cards' model with sheets of glassy state poly- α -methylstyrene alternating with sheets of lightly cross-linked polydimethylsiloxane. The layers bend under stress with viscous resistance from the accompanying interlamellar shear. The thickness of the poly- α -methylstyrene phase (h) and of the polydimethylsiloxane phase (d) are both small ($\sim 100 \text{ \AA}$) compared with the largest in-plane dimension (l), expected to be $\sim 1 \text{ }\mu\text{m}$. The difference between the models is that in case (1) the lamellae are rigidly constrained at a domain boundary at one end only so that each can bend only as a rigidly clamped cantilever, whereas in case (2) the lamellae are only loosely constrained at one end so that they can rotate and give rise to deformation by interlamellar shear. In model (1) each glassy-state lamella bends as a separate cantilever giving rise to different displacements on the top (extended) and bottom (contracted) surface of each. The glassy lamellae can only bend, therefore, if the viscous silicone phase is simultaneously sheared. Voigt retardation time (case (1)) of this model is

$$\tau_1 = \frac{3\eta l^2}{E' h d n}$$

where E' is the in-phase component of the glassy lamellar Young's modulus, η (strictly G''/ω) the viscosity of the silicone rubber phase and n the number of lamellae in the domain. Model (2) allows bending by the above scheme (with retardation time τ_1) but also allows a subsequent relaxation of stress through

Fig. 2 Temperature-independent mechanical loss factor ($\tan\delta$) and Young's modulus (E') for a benzene-cast sample in the bending mode at the frequencies shown.



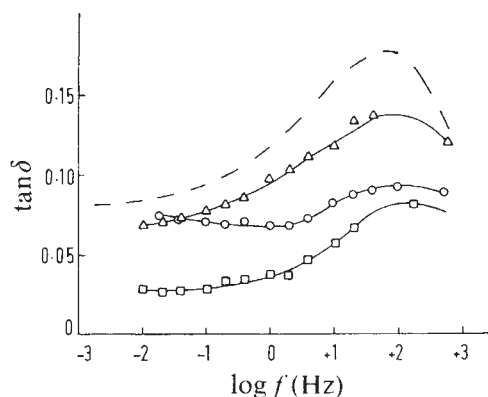


Fig. 3 Mechanical loss factor ($\tan\delta$) in the frequency plane, at 293 K, for moulded ($\square$) and benzene-cast ($\triangle$) samples in the bending mode and benzene-cast samples also in shear, in the plane of the sheet ($\circ$) and normal to the plane of the sheet ($\triangle$).

interlamellar shear with loss of the bending deformation. This gives rise to a Maxwell model situation with a relaxation time

$$\tau_2 = \frac{3l^4\eta}{5E'dh^3n^2\tan^2\phi}$$

Details of these derivations will be given by us elsewhere. Differences between relaxation and retardation times are fairly trivial because of the low relaxation strengths involved.

Selection of lamellar parameters from SAXS (small angle X-ray scattering) and volume fractions should now allow tests of the relations for τ_1 and τ_2 . For the benzene-cast sample $h = 82 \text{ \AA}$, $d = 153 \text{ \AA}$ and for the moulded sample $h = 98 \text{ \AA}$, $d = 182 \text{ \AA}$. Evaluation of l and n should be feasible by electron microscopy, but sectioning problems have so far made this intractable. From electron micrographs of similar lamellae-forming block copolymers¹¹⁻¹³ and the sharpness of the SAXS scattering peaks, we expect $l \sim 1-10 \text{ \mu m}$ and $n = 30-300$ per domain. E' for poly- α -methylstyrene has been obtained as $2.5 \times$ shear moduli values of Heijboer¹⁴ and η as $E''/3\omega$ from the data of Langley and Ferry¹⁵. We note that $(G''/\omega) = 2.3 \times 10^3 \text{ N s m}^{-2}$ at $\omega = 1$, but decreases to 7.3 N s m^{-2} at $\omega = 1,000$. We will select an approximate value of 10^3 N s m^{-2} corresponding to the centre of the present frequency range but strictly this parameter should be adjusted with frequency. Using the upper estimates for l and n we obtain frequencies of maximum damping $f_1 = 90 \text{ Hz}$ and $f_2 = 4 \text{ Hz}$. The plateau of loss at low frequencies can thus be understood as arising from a range of relaxation times from model (2) which depends on orientation. Domains with lamellae parallel to the stress direction will have $\langle \tan^2\phi \rangle \rightarrow \infty$ and $\tau_2 \rightarrow 0$ and vice versa. The peak in the data at 80–100 Hz is well predicted by the f_1 value above.

Further evidence in favour of this interpretation has been obtained by swelling the block copolymer in silicone fluid of molecular weight 2,000 and viscosity $5 \times 10^{-3} \text{ N s m}^{-2}$. The only effect of this is to increase the silicone polymer thickness d , to decrease its dynamic viscosity and to change the domain orientations to a more random situation (we will take $\phi = 45^\circ$). SAXS showed that, within experimental error, d increased as predicted from unidirectional swelling (16.3%) of the silicone polymer, giving an observed value of d of 180 Å (an increase of 17.6%). The viscosity of the final silicone phase (140 N s m^{-2}) was evaluated assuming that each fluid obeys the Doolittle equation $\ln\eta = A - B(1/f)$ with constant coefficients and simple additivity of free volume. The damping maxima (swollen system) are predicted to be at 800 Hz and 8 Hz. The experimental data in Fig. 4 give encouraging agreement, with

two clear regions of loss now apparent at $f_1 > 100 \text{ Hz}$ and $f_2 \sim 1 \text{ Hz}$.

The temperature independence of the loss processes in the bulk samples can now be recognised as a fortuitous constancy

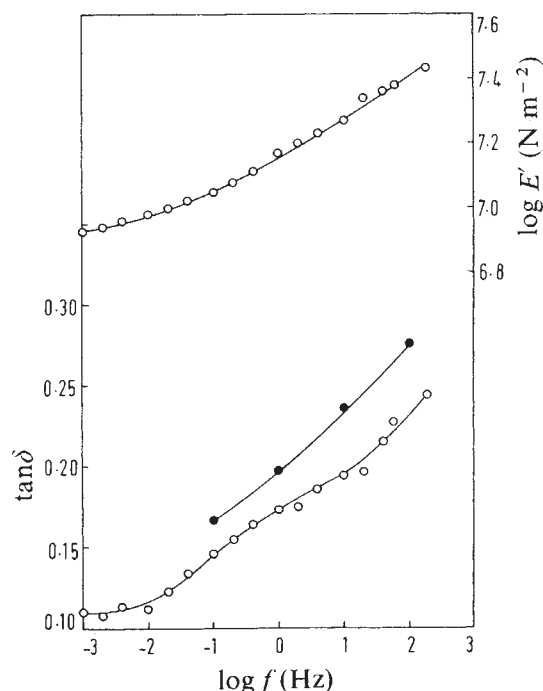


Fig. 4 Mechanical loss factor ($\tan\delta$) and Young's modulus (E') in the frequency plane at 293 K, for a benzene sample swollen with silicone oil. Loss data at 230 K ($\bullet$) now shows some temperature dependence.

in the ratio $\eta(\text{rubber})/E'(\text{glass})$ with temperature. The swollen sample does not exhibit constancy with temperature, because of a more rapid decrease of η with temperature.

The mechanisms for mechanical loss in the copolymers discussed here are expected to give rise to analogous loss processes in other lamellae-forming block copolymers and in crystalline polymers.

R. E. WETTON
W. H. TUMINELLO

Department of Chemistry,
University of Technology,
Loughborough,
Leicestershire LE11 3TU, UK

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- McCrum, N. G., Read, B. E., and Williams, G., *Anelastic and Dielectric Effects in Polymeric Solids* (Wiley, New York, 1967).
- Williams, M. L., Landel, R. F., and Ferry, J. D., *J. Am. chem. Soc.*, **77**, 3701 (1955).
- Jones, D., *Proc. IUPAC Macromolecules Conference, Aberdeen, 1973* (Butterworth, London, 1975).
- Meier, D. J., in *Block and Graft Copolymers* (edit. by Burke, J. J., and Weiss, V.), 105 (Syracuse University Press, New York, 1973).
- Bowman, I., Fielding-Russell, G. S., and Wetton, R. E., in *Polymer Systems, Deformation and Flow* (edit. by Wetton, R. E., and Whorlow, R. W.), 111 (Macmillan, London, 1968).
- Tuminello, W. H., thesis, Loughborough Univ. (1973).
- Brown, D. S., Fulcher, K. U., and Wetton, R. E., *Polymer*, **14**, 379 (1973).
- Kakudo, M., and Kasai, N., *X-ray Diffraction by Polymers* (Kodansha, Tokyo, 1972).
- Iwayanagi, S., *Rep. Progr. Polym. Phys. Japan*, **5**, 135 (1962).
- McCrum, N. G., and Morris, E. L., *Proc. R. Soc.*, **A292**, 506 (1966).
- Matsuo, M., *Jap. Plast.*, **2**, 6 (1968).
- Kampf, G., Hoffman, M., and Kromer, H., *Ber. Bunsenges. Phys. Chem.*, **74**, 851 (1970).
- Deugas, J., Keller, A., and Pedemonte, E., *Kolloid Z.-u-Z. Polym.*, **251**, 1 (1973).
- Heijboer, J., in *Physics of Non-Crystalline Solids*, 25 (North Holland, Amsterdam 1965).
- Langley, N. R., and Ferry, J. D., *Macromolecules*, **1**, 353 (1968).

Re-examination of monotony threshold hypothesis in bird song

HARTSHORNE^{1,2} has suggested that a relationship exists between the degree of versatility and continuity in bird song. According to this view, singing which is relatively continuous, with pauses between successive songs relatively short when compared with the length of the song, would tend to be highly monotonous unless successive utterances were variable in pattern.

Hartshorne lent support to his hypothesis by showing a rough correlation of versatility with continuity³. Continuity was calculated by dividing the song length by its cadence (song length + length of following silent interval). A bird with a ratio ranging from 0 to 0.20 was termed a discontinuous singer, 0.20 to 0.50 a semi-continuous singer, and 0.50 to 1.00 a continuous singer. A versatile species was originally defined as one in which "each normal individual... has a repertoire of four or more distinct songs" all or most of which appear "in no particular order" in singing bouts lasting more than 1 or 2 min⁴. A species that is repetitious, "especially if the song is brief and simple, relatively lacking in interval variety of pitch, rhythm, and so on" is non-versatile⁴. Semi-versatile species fall between the two extremes. When a species was categorised simultaneously as discontinuous, semi-continuous, or continuous, and versatile, semi-versatile, or non-versatile in a three by three table, Hartshorne found that most species fell on a diagonal connecting discontinuous-non-versatile and continuous-versatile (Table 1a).

Hartshorne exploited data obtained mainly by ear. Here we present an assessment based principally on information recorded on tape. A literature search resulted in usable data for 39 species of birds, most of which are found in North America. Data for each bird included song length, number of songs in the repertoire and intersong interval, the last being in some cases derived either for the cadence, or from estimates of number of songs per min. Additional data were found for other species—most commonly song length—but species for which all three measures were not available were excluded from consideration.

The definition of song, and therefore song length, may not be consistent from worker to worker, but each worker's own definition of song length was used even though this may introduce some ambiguity in interspecies comparison. Thus for the mockingbird, Wildenthal⁵ noted that "Hartshorne apparently measured the duration of units corresponding to the groups or phrases of the present study, and thus he included silent periods

Table 1 Data compiled by Hartshorne (a) and the data reported here (b) categorised by versatility and continuity

a	Non-continuous	Semi-continuous	Continuous
Versatile	0?	4	9
Semi-versatile	6	24	5
Non-versatile	40	11	1?

b	Non-continuous	Semi-continuous	Continuous
Versatile	10	14	3
Semi-versatile	0	6	0
Non-versatile	2	4	0

between syllables and syllable clusters in the performance time". Wildenthal noted that mockingbirds were only semi-continuous if intersyllable periods were included in the performance time; therefore Table 2 lists a weighted average of mockingbird syllable-pattern length compared with intersyllable pattern length, which gives a percentage performance time within the range Wildenthal reports as comparable to Hartshorne's figures. For other species as well, averaged figures are included when a range of values was given. In the case of five species, repertoire size was listed only as large when the number of patterns or combinations of patterns became too numerous to estimate accurately. The rose-breasted grosbeak for example may change only a few elements in each successive utterance, producing a new combination, or song, each time⁶; it also has unusually long pauses between songs (Fig. 1).

Song length for each species was divided by cadence to produce a percentage performance time. Using Spearman's rank-correlation coefficient our first test compared repertoire size and percentage performance time, which should be significantly correlated if Hartshorne's predictions are valid; however, no significant correlation was found ($r_s = 0.20$, not significant). Even a simple three by three categorisation of these data gave results markedly different from those of Hartshorne (Table 1b). Song length compared with repertoire size also was not significantly correlated ($r_s = -0.15$, not significant).

Clearly these data do not support Hartshorne's hypothesis. Possible differences in the measurement of versatility and continuity between these data and Hartshorne's require comment. By Hartshorne's original criteria, perhaps as many as 27 out of the 39 species examined would qualify as versatile, although

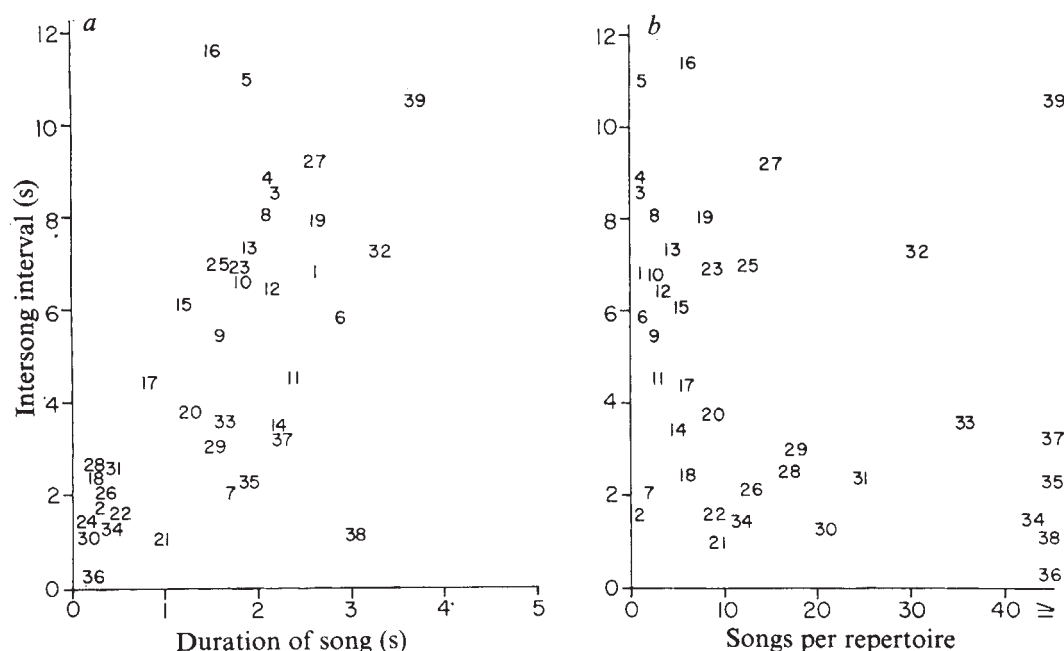


Fig. 1 Correlations of intersong intervals with duration of song (a) and size of repertoire (b). Numbers correspond to species listed in Table 2.

Table 2 Song data for various species of birds

	Species	Repertoire size (songs)	Song length	Intersong interval	Percentage of performance time	Reference
1.	Chipping sparrow (<i>Spizella passerina</i>)	1	2.61	6.79	0.28	10, 11
2.	Huttons vireo (<i>Vireo huttoni</i>)	1	0.29	1.54	0.16	12
3.	Indigo bunting (<i>Passerina cyanea</i>)	1	2.20	8.61	0.20	13
4.	Lazuli bunting (<i>Passerina amoena</i>)	1	2.13	8.79	0.20	13
5.	White-crowned sparrow (<i>Zonotrichia leucophrys</i>)	1+	1.90	11.00	0.15	14
6.	White-throated sparrow (<i>Zonotrichia albicollis</i>)	1+	2.87	5.83	0.33	15
7.	Varied bunting (<i>Passerina versicolor</i>)	2	1.70	2.00	0.46	13
8.	Chaffinch (<i>Fringilla coelebs</i>)	2+	2.10	8.07	0.21	16, 17
9.	Mexican junco (<i>Junco phaeonotus</i>)	2+	1.63	5.46	0.23	18
10.	Veery (<i>Hylocichla fuscescens</i>)	2+	1.84	6.73	0.22	19, 20
11.	Bewick's wren (<i>Thryomanes bewickii</i>)	3	2.39	4.48	0.35	20, 21
12.	Grey-cheeked thrush (<i>Hylocichla minima</i>)	3+	2.14	6.43	0.25	19, 20
13.	Painted bunting (<i>Passerina ciris</i>)	4+	1.91	7.37	0.21	13
14.	Orange-breasted bunting (<i>Passerina leclancherii</i>)	5	2.21	3.40	0.39	13
15.	Happy wren (<i>Thryothorus felix</i>)	5+	1.20	6.10	0.16	(R. N. Brown, unpublished)
16.	Brown towhee (<i>Pipilo fuscus</i>)	6	1.49	11.60	0.11	22
17.	Rufous-sided towhee (<i>Pipilo erythrophthalmus</i>)	6	0.83	4.39	0.16	23
18.	Yellow-throated vireo (<i>Vireo flavifrons</i>)	6	0.35	2.45	0.13	12
19.	Sinaloa wren (<i>Thryothorus sinaloa</i>)	8	2.60	7.95	0.25	(R. N. Brown, unpublished)
20.	Black-crested titmouse (<i>Parus atricristatus</i>)	9	1.27	3.75	0.25	24
21.	Black-throated sparrow (<i>Amphispiza bilineata</i>)	9	1.02	0.98	0.51	25
22.	Black-whiskered vireo (<i>Vireo altiloquus</i>)	9	0.51	1.57	0.25	12
23.	Cardinal (<i>Cardinalis cardinalis</i>)	9	1.80	6.90	0.21	26
24.	Gray vireo (<i>Vireo vicinior</i>)	12	0.21	1.39	0.13	12
25.	Hermit thrush (<i>Hylocichla guttata</i>)	13	1.59	6.98	0.19	19, 20
26.	Solitary vireo (Eastern) (<i>Vireo solitarius</i>)	13	0.35	2.09	0.14	12
27.	Song sparrow (<i>Melospiza melodia</i>)	15+	2.62	9.18	0.22	11, 27
28.	Solitary vireo (Western) (<i>Vireo solitarius</i>)	17	0.33	2.51	0.11	12
29.	Wood thrush (<i>Hylocichla mustelina</i>)	18	1.56	3.04	0.34	19, 20
30.	Yellow-green vireo (<i>Vireo flavoviridis</i>)	21	0.17	1.29	0.12	12
31.	Philadelphia vireo (<i>Vireo philadelphicus</i>)	25	0.40	2.47	0.14	12
32.	Willow warbler (<i>Phylloscopus trochilus</i>)	31	3.31	7.27	0.31	28
33.	Carolina wren (<i>Thryothorus ludovicianus</i>)	36	1.66	3.59	0.32	29
34.	Red-eyed vireo (<i>Vireo olivaceus</i>)	43	0.35	1.42	0.20	12
35.	American robin (<i>Turdus migratorius</i>)	L	1.93	2.28	0.46	(C. W. D. and R. E. L. unpublished)
36.	Catbird (<i>Dumetella carolinensis</i>)	L	0.27	0.19	0.59	30
37.	European robin (<i>Erithacus rubecula</i>)	L	2.18	3.27	0.40	31
38.	Mockingbird (<i>Mimus polyglottos</i>)	L	3.06	1.10	0.74	5
39.	Rose-breasted grosbeak (<i>Pheucticus ludovicianus</i>)	L	3.70	10.50	0.26	6

Solitary vireo is listed twice as the Eastern and Western populations have different song data.

our list does not disqualify birds showing only "eventual" versatility—birds such as the cardinal which, though having a large repertoire, may sing only one or two songs for a period of time before changing to others. Some variability (for example, song length, number of repeated elements) is found even in the single song type of the chipping sparrow⁷, and Hartshorne, discussing Hutton's vireo as a possible exception to his hypothesis suggests that a slight "change of key" may render the single, repetitive song less monotonous over time. Nevertheless ranking versatility by measuring repertoire size seems a reasonable approach, particularly given the ability of spectrographic analysis to detect differences between song patterns.

The use of average values for song length and intersong interval may also conflict with Hartshorne, who cites instances of unusually continuous singing in versatile birds that normally sing quite discontinuously. There is no reason to suppose, however, that non-versatile discontinuous singers might not show similar examples of more rapid singing. If the monotony threshold hypothesis is to have practical predictive value, it should apply over the broad range of bird songs making up our averaged figures.

In spite of the lack of significance in the two comparisons, two significant correlations in the data were found. Song length and intersong interval gave a strong positive correlation ($r_s = 0.60$, $P < 0.05$), as shown in Fig. 2. This suggests that each species in fact tends to sing in a manner that uses a sound-silence ratio similar to other species, but using a different rhythm, or cadence. These differences in rhythm may be an important quality differentiating one bird's song from another. The same sort of relationship of sound to silence can be found in syllable structure of the cardinal—short syllables being followed by short pauses and longer syllables being followed by longer pauses⁸. Both sorts of data emphasise the fundamental rhythmical quality of song, and it is interesting to speculate that this quality may in part be related to the physiology of vocalisation in birds. The process of respiration perhaps places constraints on song and syllable lengths as well as the intervals between them⁹. Alternatively, these temporal rhythmical relationships may be related to the effectiveness of song in overcoming environmental noise. Repetitive songs, or elements of songs produced at predictable intervals, may provide a high degree of redundancy which would provide for more effective reception by listeners.

Finally, a negative correlation between interval length and repertoire size ($r_s = -0.39$, $P < 0.05$, Fig. 1) may be viewed as supporting Hartshorne's basic notion of temporal differences between versatile and non-versatile species, while suggesting that percentage of performance time is not a good measure of it. Longer intervals between songs in more versatile birds might easily lead a field observer to suppose that continuity, rather than cadence, correlates with versatility. In view of our findings, a relationship between versatility and percentage of performance time is by no means general or predictable.

CHARLES W. DOBSON
ROBERT E. LEMON

Department of Biology,
McGill University,
PO Box 6070, Montreal, Quebec, Canada H3C 3G1

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- ¹ Hartshorne, C. *Auk*, **73**, 176–192 (1956).
- ² Hartshorne, C., *Born to Sing* (Indiana University Press, Bloomington, 1973).
- ³ *Ibid.*, 125.
- ⁴ Hartshorne, *op. cit.*, 176.
- ⁵ Wildenthal, J. L., *Auk*, **82**, 161–189; 167–168 (1965).
- ⁶ Lemon, R. E., and Chatfield, C., *Anim. Behav.*, **21**, 28–44 (1973).
- ⁷ Marler, P., and Isaac, D., *Condor*, **62**, 124–135 (1960).
- ⁸ Lemon, R. E., *Z. Tierpsychol.* (in the press).
- ⁹ Lasiewski, R. C., in *Avian Biology* 2 (edit. by Farner, D. S., and King, J. R.), 287–342 (Academic, New York, 1972).
- ¹⁰ Borror, D. J., *Ohio J. Sci.*, **59**, 347–56 (1959).
- ¹¹ Reynard, G. B., *Living Bird*, **2**, 139–148 (1963).
- ¹² Borror, D. J., *Condor*, **74**, 80–86 (1972).
- ¹³ Thompson, W. L., *Behaviour*, **31**, 261–287 (1968).
- ¹⁴ Marler, P., and Tamura, M., *Condor*, **64**, 368–376 (1962).
- ¹⁵ Borror, D. J., and Gunn, W. W. H., *Auk*, **82**, 26–47 (1965).
- ¹⁶ Barber, D. R., *Nature*, **163**, 129 (1959).
- ¹⁷ Marler, P., *Ibis*, **94**, 458–472 (1952).
- ¹⁸ Marler, P., and Isaac, D., *Wilson Bull.*, **73**, 193–206 (1961).

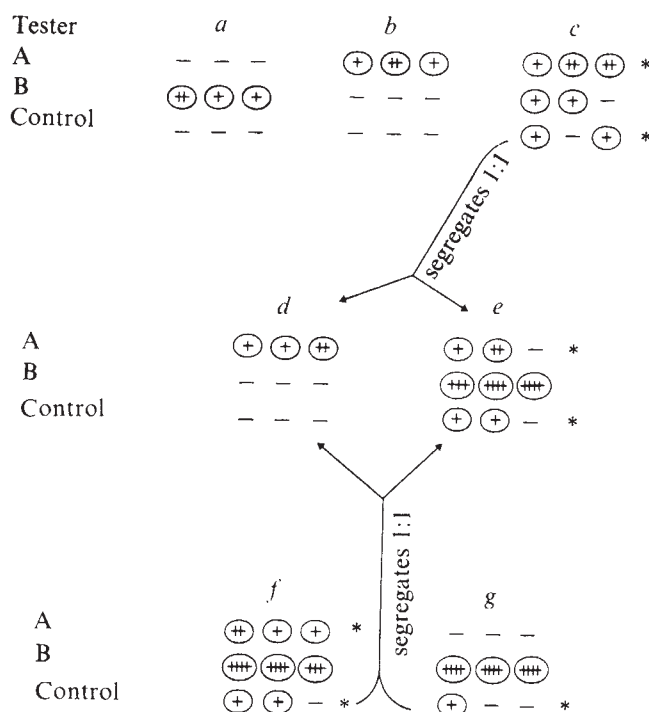
- ¹⁹ Stein, R. C., *Auk*, **73**, 507–512 (1956).
- ²⁰ Borror, D. J., *Ohio J. Sci.*, **64**, 195–207 (1964).
- ²¹ Fish, W. R., *Condor*, **55**, 250–257 (1953).
- ²² Marler, P., and Isaac, D., *Auk*, **77**, 433–444 (1960).
- ²³ Kroodsma, D. E., *Condor*, **73**, 303–308 (1971).
- ²⁴ Lemon, R. E., *Can. J. Zool.*, **46**, 1163–1169 (1968).
- ²⁵ Heckenlively, D. B., *Condor*, **73**, 24–36 (1970).
- ²⁶ Lemon, R. E., and Herzog, A., *Condor*, **71**, 1–15 (1969).
- ²⁷ Mulligan, J. A., *Proc. 12th int. Orn. Congr.*, 272–284 (1963).
- ²⁸ Schubert, M., *J. Orn.*, **108**, 265–294 (1967).
- ²⁹ Borror, D. J., *Auk*, **73**, 211–229 (1956).
- ³⁰ Thompson, W. L., and Jane, P. L., *Jack-Pine Warbler*, **47**, 115–125 (1969).
- ³¹ Bremond, J., *C. r. hebdom. Séanc. Acad. Sci. Paris*, **259**, 3365–3366 (1964).

Highly fertile form of the aggressive strain of *Ceratocystis ulmi*

THE ability to recognise the aggressive and non-aggressive strains of *Ceratocystis ulmi* in culture¹ has enabled us to survey the *C. ulmi* population in the field and thereby monitor any variation that might indicate a change in the course of the present epidemic of Dutch elm disease in Britain. When sampling from diseased elm twigs in the outbreak areas, we have found that while most wild isolates can be assigned to the two strains, there also exist in low frequency (about 2%) isolates with some affinity to the 'fluffy' aggressive strain but which produce small black-brown sclerotium-like bodies throughout the culture and are dark centred because of the presence of brown pigment. We show here that these isolates are a highly fertile form of the aggressive strain, with a special function in the perithecial, or sexual stage of the fungus.

Production of perithecia in *C. ulmi* usually requires the pairing of the two mating or compatibility types, A and B (ref. 2). During routine screening of wild isolates of the aggressive strain for compatibility type, after the method of Holmes³, autoclaved

Fig. 1 Mating reactions of *C. ulmi* isolated on elm twigs. Each diagram (a–g) represents the reaction pattern of an unknown isolate when tested against a standard non-aggressive A type, an aggressive B type, and on its own as a control, with three replicate twigs in each case. Perithecia after 1 month at 18 °C: —, nil; +, rare; ++, occasional; +++, frequent; +++++, abundant. Reaction patterns: a, normal A type reaction (as found with non-aggressive isolates); b, normal B type (as found with non-aggressive and aggressive isolates); c, aggressive B type with pseudoselfing at *; d, F₁ fluffy B type; e, F₁ proto A type, with pseudoselfing at *; f, g, wild proto A types, with pseudoselfing at *.



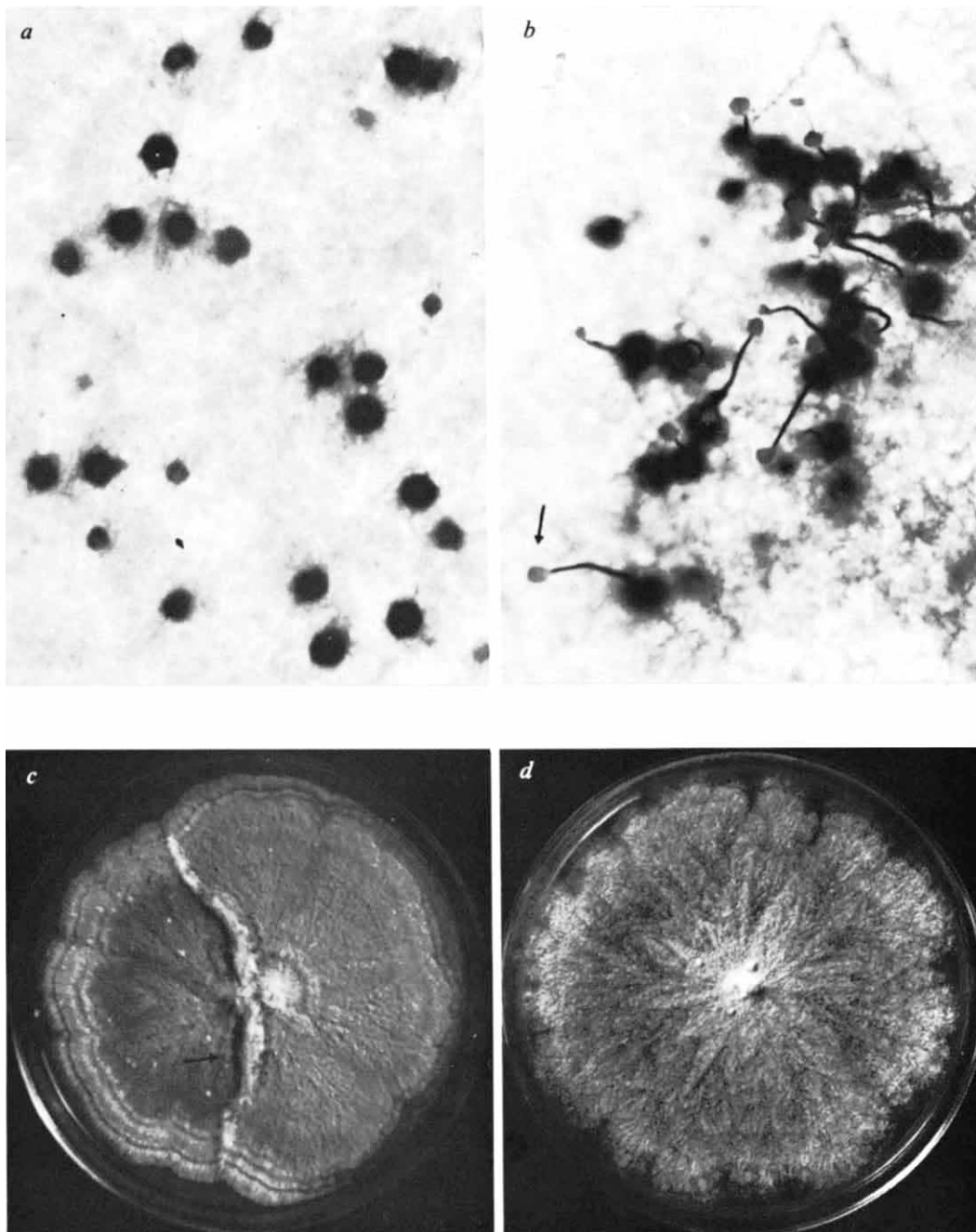


Fig. 2 Perithecium formation by *C. ulmi* on malt agar. *a*, Protoperithecia formed in a culture of a proto isolate ($\times$ about 65). *b*, Mature perithecia formed at the junction of a proto and a fluffy isolate, showing typical long perithecial necks and oozing blobs of ascospores (arrowed; $\times$ about 25). *c*, Formation of a black line of perithecia (arrowed) and a white felty zone line at the junction of a dark pigmented proto (left) and a fluffy (right) isolate. Culture incubated for 12 d in darkness at 18 °C, followed by daylight. *d*, Wild proto isolate S6. Culture incubated in darkness at 18 °C.

peeled elm twigs were dipped first in shake cultures of the unknown isolate, and then after incubation for 1 week at 18 °C, dipped again in a shake culture of a standard A or B tester strain. Control twigs were dipped in the unknown isolate only. No isolate gave a type A reaction (Fig. 1*a*) and most gave a clear-cut type B reaction, forming perithecia with the A tester only (Fig. 1*b*). Some isolates, however, while behaving as type B, occasionally formed scattered perithecia on the control twigs (Fig. 1*c*). As all isolates tested were originally derived from single spores, this 'selfing' phenomenon was particularly curious. Ascospores from such 'selfed' perithecia of three isolates, G11, G29 and S46 were therefore spread on malt agar, and retained on the bench top for observation. After 2 weeks the resulting colonies were seen to be producing abundant fertile perithecia (Fig. 2*b*), an interesting development as perithecia have normally been obtained only on sterilised elm bark or sapwood. The perithecia seemed to be forming at the junction of light and dark coloured colonies as a result of the maturation of numerous small spherical black-brown 'protoperithecia' (about 30–55 μ m, Fig. 2*a*).

To investigate the phenomenon in more detail, fifty single ascospore germings picked off from further spreads of isolate G11 were examined for growth rate and cultural characters on 2% Oxoid Malt Extract Agar at 18 °C. They varied significantly in growth rate, with a range which fell within that normally encountered in aggressive isolates (Fig. 3*a*). On the basis of culture morphology, however, they fell into two groups in a nearly 1:1 ratio. Isolates in one group resembled the parent aggressive isolate in their fluffy morphology¹. Isolates in the other group had some fluffy characteristics but, in contrast, were dark pigmented and also formed considerable numbers of protoperithecia. These isolates were therefore called protoperithecial ('proto'). The mean growth rate of the proto progeny (4.05) was significantly lower ($P < 0.01$) than that of the fluffy progeny (4.25). Ascospore progeny of two other isolates, S46 and G29, also fell into the two groups. With G29 progeny behaviour was similar to that for G11 but in S46 complete separation of the proto and fluffy progeny occurred for growth rate, with the proto isolates growing very much more slowly (Fig. 3*b*). When F_2 ascospores of G11 and S46 were sampled

from perithecia developing in the F_1 ascospore spreads, the process repeated itself, the progeny again separating into proto and fluffy groups.

As Fig. 3 shows, there was considerable variation in growth rate within the two groups. Variation also occurred in morphology, for example, in the pigmentation and abundance of protoperithecia in the proto form, and in the appearance of the aerial mycelium in the fluffy. This variation was particularly marked in the F_4 line of G11 derived by mixing and spreading the ascospores of 20 perithecia from first the F_2 and then the F_3 lines. Several new cultural types occurred within both groups.

The presence of perithecia at the junction of light and dark colonies suggested the involvement of both proto and fluffy progeny types in perithecial formation. A fluffy progeny isolate was therefore paired with its proto sibling on malt agar. Where the two colonies met, a zone of dense white felty mycelium was formed, and later a line of fertile perithecia developed (Fig. 2c). The ascospores again segregated 1:1 for the two types. Whatever their origin, any combination of proto $\times$ fluffy progeny isolates formed perithecia, but fluffy $\times$ fluffy and proto $\times$ proto pairings did not. Furthermore, any wild fluffy isolate behaved in the same way as the fluffy progeny isolates. This suggested either stimulation of selfing, or cross fertilisation between the fluffy and proto types. When a proto progeny culture was seeded with conidia of a fluffy isolate, perithecia sometimes developed at the site of conidial application. This did not happen when a fluffy progeny isolate was seeded with a proto isolate, indicating that in a proto $\times$ fluffy pairing, it is the protoperithecia of the proto culture which mature. Ability of the fluffy to fertilise the proto was demonstrated when ascospores resulting from the seeding of protoperithecia with a fluffy isolate tolerant to the fungicide MBC (ref. 4) segregated for MBC tolerance.

These results suggested a difference in compatibility type between fluffy and proto isolates. In tests of F_1 progeny of G11 and S46 on elm twigs the fluffy isolates gave a normal B-type reaction (Fig. 1d). Proto isolates gave a very strong A-type reaction and also formed numerous perithecia in patches on the control and A tester twigs (Fig. 1e). This showed that the proto is a highly fertile form of the A compatibility type and has, in addition, a marked tendency to 'self'.

It was already evident that proto progeny obtained from 'selfed' aggressive isolates closely resembled in morphology the dark sclerotial types found occasionally in nature (Fig. 2d). This similarity was confirmed when: (1) pairings between dark sclerotial (hereafter called 'wild proto') isolates and fluffy isolates on malt agar resulted in the formation of fertile perithecia; (2) progeny from these perithecia segregated 1:1 for proto and fluffy; (3) wild proto isolates showed a strong A compatibility reaction with a tendency to 'self' on control twigs (Fig. 1f and g); and (4) ascospores from such 'selfed' perithecia segregated 1:1 for proto and fluffy. One such wild proto isolate (T7) with a slow growth rate (about 2.8 mm d^{-1}) produced typical fast-growing fluffy progeny (range of five isolates $3.1\text{--}3.8 \text{ mm d}^{-1}$) as well as slower growing proto progeny (range of five isolates $2.7\text{--}3.2 \text{ mm d}^{-1}$).

This indicates that the fluffy type can be derived from a proto parent as well as the proto type from a fluffy parent and highlights the need to understand the mechanism by which this occurs. The evidence cannot be reconciled with the non-parental type having arisen by true selfing in a diploid or polysomic line, a situation which would in any case be most unusual in an ascomycete. Indeed the consistent 1:1 segregation of the two types suggests instead a single gene difference in a haploid. In this situation, the non-parental genotype could either arise by mutation or be pre-existing within the parent as a component of a heterokaryon. Because the isolates examined were derived from single uninucleate conidia⁵, and because successively 'singled' proto isolates 'self' on twigs, the origin of the phenomenon in heterokaryosis seems unlikely. On present evidence we prefer the hypothesis that within the fluffy B type mutation to proto A type occurs at a single locus. Proto nuclei arising within the mycelium of the vegetatively growing fluffy isolate on malt

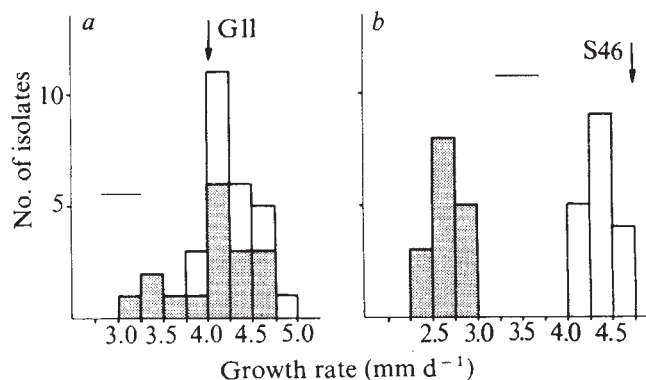


Fig. 3 Growth rate distribution of F_1 progeny derived from 'selfed' perithecia of aggressive isolates of *C. ulmi*. a, Progeny of isolate G11, b, Progeny of isolate S46. Proto progeny stippled, fluffy progeny white. Arrows show parental growth rates, and bars indicate least significant difference between isolates at $P < 0.05$.

agar are normally suppressed. When, however, an elm twig is dipped in a shake culture (in which a yeast-like budding phase occurs) spores containing proto nuclei are able to develop, resulting in localised A $\times$ B fertilisation and maturation of perithecia. Resulting ascospores segregate 1:1 mutant (proto): wild type (fluffy). With this 'pseudoselfing', background variation such as growth rate among both proto and fluffy progeny could be due to segregation of mutations at other loci, and perhaps to some cytoplasmic variation. A similar explanation based on the reverse mutation could apply to the origin of the fluffy from the proto type.

Preliminary data suggest that the postulated proto locus has some influence on pathogenicity as well as on growth rate and culture morphology. An inoculation experiment on 5-yr-old clonal English elm, *Ulmus procera*, using techniques described earlier¹ with four replicates, showed that while three wild proto isolates caused very much more defoliation than two non-aggressive isolates, they caused significantly less ($P < 0.05$) than twelve wild fluffy aggressive isolates; mean percentage defoliation at 8 weeks being 2.3% for the non-aggressive, 50.8% for the proto and 75.9% for the fluffy isolates.

The occurrence of the protoperithecial form of the aggressive strain, and the phenomenon of pseudoselfing, reveal previously unknown properties in *C. ulmi* with considerable ecological implications. Perithecia occur naturally in beetle breeding galleries within the bark of diseased elms and the ascospores, together with asexual spores, are thought to be carried by the newly emerging beetles in the spring. Until now it has been assumed that for perithecial formation the absence of an aggressive A type in Britain would mean that the aggressive strain would have to hybridise with the non-aggressive strain, leading to a decline in pathogenicity⁶. It is now clear that this is not the case and that an aggressive B type could undergo pseudoselfing to produce two progeny genotypes, one the fluffy B form, close to the parent itself, and the other the highly fertile proto A form. Furthermore, in elm bark the latter would presumably produce abundant protoperithecia, which would be available for fertilisation, whether by pseudoselfing or by outcrossing with available B types. It seems that the aggressive strain could thus maintain itself even where perithecial formation rather than asexual reproduction was at a premium. The natural balance between the two forms would presumably depend on the relative selection pressures towards such attributes as perithecial formation, saprophytic ability within bark and pathogenicity.

The role of the proto form in the present epidemic is being investigated. The low frequency of proto isolates in twig samples suggests that in the pathogenic phase of the fungus this form is presently less fit than the fluffy form. Some perithecia from bark in the main outbreak areas have, however, produced equal numbers of fluffy and proto progeny. Furthermore, a reinvesti-

gation of the ascospore progeny from the perithecia found in beetle galleries of the imported rock elm logs examined in January 1973 (ref. 7) has shown that these too consist of proto and fluffy forms. Thus these perithecia must have resulted from proto $\times$ fluffy outcrossing or pseudoselfing.

Our laboratory experiments have shown that pseudoselfing and proto $\times$ fluffy outcrossing can result in significant variation among progeny. In nature this could lead to the appearance of new genotypes, and in a situation in which selection for aggressiveness were relaxed, might well contribute to a decline in the pathogenicity of the present *C. ulmi* population and thus of the epidemic.

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C. M. BRASIER
J. N. GIBBS

Forestry Commission Research Station,
Alice Holt Lodge, Farnham, Surrey, UK

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- ¹ Gibbs, J. N., and Brasier, C. M., *Nature*, **241**, 381-383 (1973).
- ² Shafer, T., and Liming, O. N., *Phytopathology*, **40**, 1035-1042 (1950).
- ³ Holmes, F. W., *Phytopathology*, **62**, 495 (1972).
- ⁴ Brasier, C. M., and Gibbs, J. N., *Ann. appl. Biol.*, **80**, 231-235 (1975).
- ⁵ Sansome, E., and Brasier, C. M., *Trans. Br. mycol. Soc.*, **61**, 588-590 (1974).
- ⁶ Brasier, C. M., and Gibbs, J. N., *Ann. appl. Biol.* (in the press).
- ⁷ Brasier, C. M., and Gibbs, J. N., *Nature*, **242**, 607-609 (1973).

Morphine withdrawal response and central cholinergic activity

MOST of the different neurotransmitter systems have been implicated in opiate withdrawal. Frederickson and Pinsky¹ suggested a primary role for acetylcholine (ACh) in both the development and the expression of dependence on morphine but the specific role of cholinergic mechanisms in opiate withdrawal is not yet clear and deserves further attention. The hypothesis of the role of ACh in the expression of withdrawal phenomena, on which most earlier experiments were based, was that of the 'cholinergic excess', first postulated by Paton². Tests of this hypothesis in various laboratories have, however, produced conflicting results and thus confusion rather than clarification of the role of cholinergic mechanisms in the withdrawal syndrome³⁻⁹. Unfortunately, many such studies have neglected to differentiate between peripheral interference with expression and central interference with initiation of the signs of withdrawal by the various drugs studied. Our experiments were designed to eliminate this particular factor, which has contributed to much of the conflict. The results clearly illustrate the confounding effect of failure to separate peripheral from central cholinergic effects and suggest that the exact opposite of the cholinergic excess theory is the actual nature of the central cholinergic role in the morphine withdrawal syndrome.

Male albino rats (Sprague-Dawley, Harlan, 140-160 g) were made morphine-dependent by the administration of a slowly absorbed suspension of morphine base in mannide monooleate (300 mg kg⁻¹, subcutaneously) as described previously^{9,10}. Withdrawal was precipitated 48 h later, which is the approximate time of peak withdrawal response¹⁰, by the injection of naloxone (0.03-3.0 mg kg⁻¹, subcutaneously) and withdrawal severity was assessed by a point-scoring technique modified slightly from that described previously¹⁰ by weighting the signs. The range of scores possible for each sign is given in the legend to Fig. 1. In the present experiments the withdrawal signs scored were grouped before analysis as follows. Group 1: those

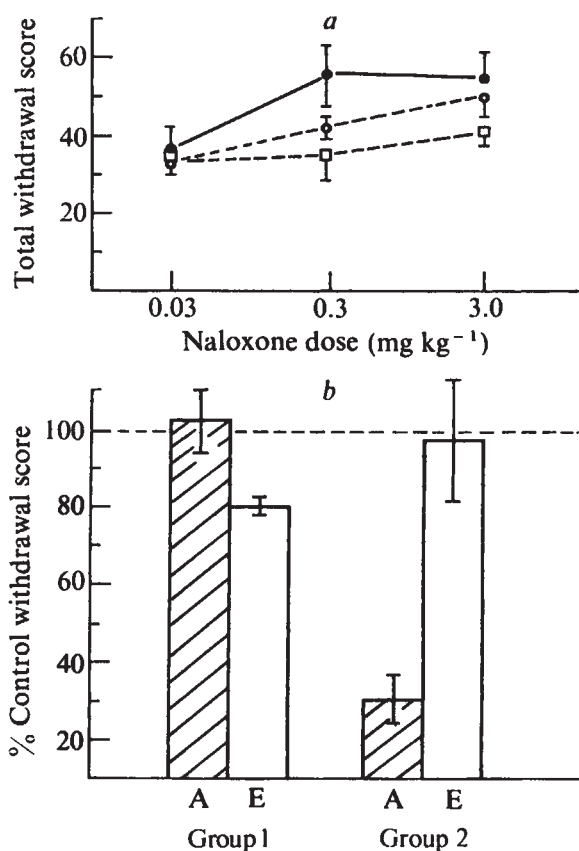


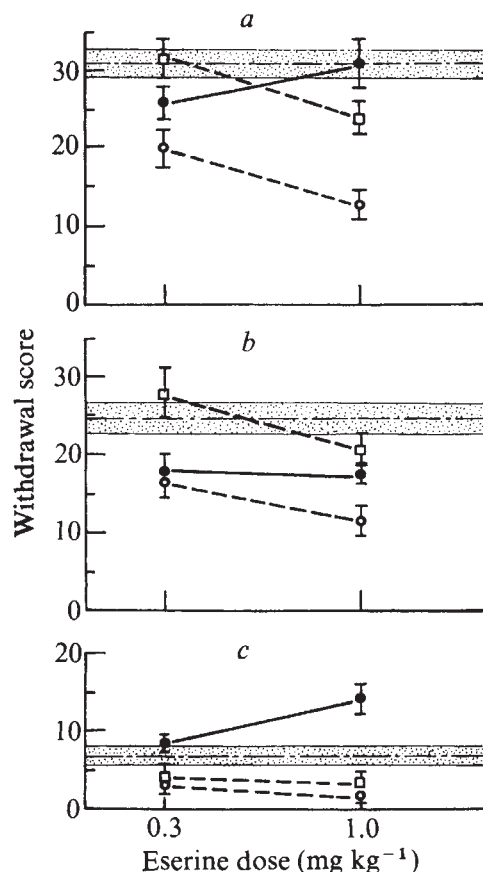
Fig. 1 *a*, The effects of eserine sulphate (0.25 mg kg⁻¹, intraperitoneally, (●)) and atropine sulphate (5 mg kg⁻¹, intraperitoneally, (□)) on the severity of the naloxone-induced withdrawal response of morphine-dependent rats. The points refer to the means (± s.e.) of the total withdrawal score per animal (*n* = 6 rats per point). The signs scored, with the range of possible scores for each sign, follow; group 1 signs: wet dog shakes (0-12), escape jumping (0-36), escape digging (0-12), yawning (0-12), ptosis (0-8), penile erection (0-12), irritability to handling (0-6), reaction to poking with sharp object (0-12), restless activity (0-8); group 2 signs: rhinorrhea (0-8), lacrimation (0-12), salivation (0-18), diarrhoea (0-8), seminal emission (0-8). Control animals (●) received saline before naloxone. *b*, Effects of eserine sulphate (0.25 mg kg⁻¹, open bars) and atropine sulphate (5 mg kg⁻¹, hatched bars) on group 1 as compared with group 2 signs. The data are presented as percentages (mean ± s.e.) of 100%. The scores for the group 1 signs and the scores for the group 2 signs were determined separately. The data presented in this figure were obtained from a total of 54 rats, 18 per treatment mode. A, Atropine sulphate; E, eserine sulphate.

signs with a strong voluntary component which were found to be influenced by muscarinic or antimuscarinic drugs by action in the central nervous system; and group 2: those signs with a strong autonomic or peripheral parasympathetic component whose peripheral expression was found to be influenced by muscarinic or antimuscarinic drugs by action in the periphery. The actual signs used for each analysis are given in the legend to each figure. Eserine sulphate and atropine sulphate were injected intraperitoneally 30 min before the challenge with naloxone. When combined treatment was used, either atropine sulphate or atropine methylnitrate was injected intraperitoneally immediately before eserine sulphate.

The total withdrawal score precipitated by various doses of naloxone (0.03-3.0 mg kg⁻¹, subcutaneously) was slightly diminished by treatment with eserine at 0.25 mg kg⁻¹ and similarly with atropine at 5 mg kg⁻¹ (Fig. 1*a*). The apparent paradox of a cholinergic agent, eserine, and an anticholinergic agent, atropine, producing similar effects can be explained if the different possible sites of action are considered. Thus, when the withdrawal signs were separated as described above, it was

apparent atropine did not reduce the severity of the group 1 signs but markedly reduced the score for group 2 signs, many of which are parasympathetic in nature (Fig. 1b). Thus atropine seems to reduce withdrawal severity merely by blocking the peripheral expression of the subset of signs with a strong peripheral parasympathetic component. Conversely, eserine, at the dose used, had no effect on the peripheral signs but did reduce the severity of central signs (Fig. 1b). These results predict that the combination of a central cholinergic agent with peripheral cholinergic blockade should reduce the withdrawal response very effectively. Thus, in the presence of methylatropine, eserine should effectively alleviate overall withdrawal severity. Methylatropine blocks muscarinic receptors in the periphery but does not readily penetrate the blood-brain barrier. We did, in fact, observe a marked dose-dependent reduction in withdrawal severity of morphine-dependent rats after combined treatment with eserine and methylatropine (Fig. 2a). Eserine alone did not have such an effect. The importance of the central cholinergic activity of eserine in reducing the withdrawal response became evident when the effects of combined treatment with eserine and atropine sulphate were examined. Atropine sulphate should have the same actions peripherally as methylatropine, yet, the combination of eserine and atropine did not have the same ameliorating effect on withdrawal as did the combination with methylatropine (Fig. 2a). This is interpreted as attributable to antagonism of the central cholinergic actions of eserine by atropine. An examination of group 1 and group 2 signs separately further confirmed this interpretation (Fig. 2b, c).

Fig. 2 The effects of eserine sulphate (0.3 and 1.0 mg kg⁻¹ subcutaneously) alone ● and in combination with atropine methyl-nitrate ○ or atropine sulphate □ on (a), total withdrawal score, (b), group 1 withdrawal score; and (c), group 2 withdrawal score. The stippled regions on each graph represent the control withdrawal score (mean ± s.e.). The signs used for the total, group 1 and group 2 scores were identical to those listed in the legend to Fig. 1. The data presented in this figure were obtained using 42 rats, 6 rats per point. Naloxone was injected at 0.3 mg kg⁻¹ subcutaneously to precipitate withdrawal. The dose of atropine methyl-nitrate and atropine sulphate used in these experiments was 5 mg kg⁻¹ intraperitoneally.



The higher dose of eserine increased the severity of the group 2 signs and both atropine and methylatropine blocked this effect (Fig. 2c). Thus eserine seems to increase the severity of group 2 signs by a muscarinic action in the periphery. Both doses of eserine reduced the severity of the group 1 signs (Fig. 2b). Atropine blocked this effect; methylatropine did not, but in fact increased the beneficial effect of the higher dose of eserine. These interactions clearly illustrate that eserine must alleviate the severity of group 1 signs by a muscarinic action in the central nervous system.

These results provide further evidence for an important role for acetylcholine in morphine withdrawal but do not support the hypothesis^{2,11,12} of a cholinergic crisis resulting from excessive release of ACh on to supersensitive receptors. On the contrary, the specific derangement in central cholinergic function which occurs after the administration of naloxone to a morphine-dependent animal seems to be a cholinergic deficit. Thus when the interference from cholinergic actions in the periphery is eliminated by methylatropine, eserine relieves the withdrawal response, presumably because of an increase in central muscarinic activity, as it is blocked by atropine.

Frederickson and Pinsky¹ reported that the syndrome which occurs on abstinence morphine withdrawal is biphasic in nature. The early phase seems to coincide with a cholinergic deficit since it is potentiated by anticholinergic treatment, while the later phase seems to have a cholinergic component, since its severity is reduced by treatment with anticholinergic drugs. Labrecque and Domino¹³ have reported a biphasic effect of naloxone also on cortical ACh release in morphine-dependent cats. ACh output was decreased at 15 min after 1.0 mg kg⁻¹ of naloxone while an increase was found at 45–105 min. Thus the naloxone-precipitated withdrawal response seems to be an accelerated and accentuated correlate of the spontaneous withdrawal syndrome. The studies of ACh release should be repeated on rats, but it is interesting that the withdrawal response precipitated by naloxone, reported here as being a result of a central cholinergic deficit, occurred dramatically within the first 15 min of naloxone injection, corresponding to the time the decrease in output of cortical ACh was observed by Labrecque and Domino¹³.

In conclusion, the present results provide an explanation for the previous conflicting reports³⁻⁹ of the effects of cholinergic and anticholinergic drugs on opiate withdrawal, since either an increase or a decrease in withdrawal severity could be expected, with either a cholinergic or an anticholinergic agent, depending on the sign or signs examined. The early phase of the opiate withdrawal response seems to correspond to a central cholinergic deficit and I suggest the most effective alleviation of withdrawal stress requires the unique combination of a central muscarinic action plus a peripheral muscarinic blockade.

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ROBERT C. A. FREDERICKSON

Department of Pharmacology,
The Lilly Research Laboratories,
Eli Lilly and Company,
Indianapolis, Indiana 46206

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- Frederickson, R. C. A., and Pinsky, C., *J. Pharmac. exp. Ther.* **193**, 44–55 (1975).
- Paton, W. D. M., *Can. J. Biochem. Physiol.*, **41**, 2637 (1963).
- Fuentes, V. O., thesis, Univ. Nottingham (1970).
- Grumbach, L., *Fedn Proc.*, **28**, 262 (1969).
- Kolb, L., and Himmelsbach, C. K., *Am. J. Psychiat.*, **94**, 759 (1938).
- Rhamken, I. F., *Psychopharmac. Abs.*, **7**, 12 (1968).
- Bhargava, H. N., and Way, E. L., *J. Pharmac. exp. Ther.*, **183**, 31 (1972).
- Pinsky, C., Frederickson, R. C. A., and Vazquez, A. J., *Nature*, **242**, 59 (1973).
- Collier, H. O. J., Francis, D. L., and Schneider, C., *Nature*, **237**, 220 (1972).
- Frederickson, R. C. A., and Smits, S. E., *Res. Comm. chem. Path. Pharmac.*, **5**, 867 (1973).
- Collier, H. O. J., *Nature*, **220**, 228 (1968).
- Collier, H. O. J., in *Scientific Basis of Drug Dependence* (edit. by Steinberg, H.), 49–66 (Churchill, London, 1969).
- Labrecque, G., and Domino, E. F., *J. Pharmac. exp. Ther.*, **191**, 189 (1974).

Low levels of photoreactivating enzyme in xeroderma pigmentosum variants

XERODERMA pigmentosum (XP) is a recessive, hereditary disease in which patients develop malignancies on areas of

Table 1 PRE and pyruvate kinase levels in normal and XP variant cells

Cell line	Pyruvate kinase* activity (% normal)	PRE activity† (% normal)
Normal		
HESM	100	100
Variants		
XP4BE	81.4	11
XP13BE	40.6	9
XP7TA	104	4
XP30RO	80.2	56

*Determined by the method of Ibsen and Trippet¹¹.

†Cells were grown collected and assayed as described previously^{5,9}. Protein concentrations were determined by the method of Lowry¹². Typical levels of enzyme activity for normal cells were 600–620 pmol mg⁻¹ min⁻¹. All values were the average of at least three independent determinations except for XP30RO, which had two.

skin exposed to sunlight¹. Although fibroblasts from most XP individuals are deficient in excision repair of damage to DNA (classical XP), those from a few individuals (XP variants) having the usual clinical signs of XP seem to have normal excision capacity². Photoreactivation is a DNA repair process in which the photoreactivating enzyme (PRE) monomerises pyrimidine dimers induced by ultraviolet light³. As PRE is present in normal human cells⁴, but only at reduced levels in classical XP cells⁵, we thought that the XP variants may also be defective in PRE activity. We therefore measured this activity in fibroblasts from four XP variants (Table 1) and found that Variants XP30RO, XP4BE, XP13BE, and XP7TA have 56, 11, 9, and 4% of the normal level of PRE activity, respectively.

Are the lower levels of PRE an intrinsic property of the cells or an artefact of culture or assay conditions? The high levels of PRE activity in the normal cells have been shown to be neither an artefact of mycoplasma infection of the normal lines nor of age of the cell donor at biopsy⁵. We have also examined the possibility that the low PRE activity in XP variants may result from: systematic loss of enzyme activity with culture age; unfavourable enzyme assay conditions; presence of inhibitors of PRE activity; or slower growth rates of the XP cells leading to general enzyme depression. We found, however, no systematic decrease in PRE activity throughout 10 cell passages; neither an increase nor decrease in pH or ionic strength, nor the omission of Mg²⁺ from the assay mixture, increased the apparent enzyme activity in extracts of XP7TA; mixing experiments indicated that the cell extracts of XP7TA had no effect on the activity of the normal human enzyme; and pyruvate kinase (chosen as the representative enzyme of cellular metabolism other than DNA repair) levels were 40.6, 81.4, 104 and 80.2% of the normal level, for XP13BE, XP4BE, XP7TA and XP30RO, respectively. We conclude that these factors do not play a major part in determining the level of PRE activity measured in our assay. These data imply that the observed low levels of activity were not

artefacts of culture or assay conditions, and may contribute to the depression of total DNA repair capacity in XP variants.

What other processes must be considered in determining the total cellular repair capacity? In addition to photoreactivation, cells have two other pathways for repair of ultraviolet-induced damage to DNA: excision repair⁶ and postreplication repair⁷. Excision repair and photoreactivation act principally as prereplication error-correcting pathways, whereas postreplication repair works to seal gaps presumably generated opposite damaged bases during DNA replication. One measure of cellular excision repair ability is unscheduled DNA synthesis, the ultraviolet-induced incorporation of ³H-thymidine into nuclei of non-S-phase cells. XP4BE, XP13BE, XP7TA and XP30RO can carry out excision repair at almost normal levels (Table 2). Although quantitation of postreplication repair is difficult, Lehman *et al.*⁸ have shown that 1 h after ultraviolet irradiation, the DNA of XP variants is much smaller (and thus contains more unrepaired gaps) than that from normal cells. The data of Lehman *et al.*⁸ for the relative sizes of the DNAs at 1 h postirradiation are shown in Table 2. PRE activity (Table 2) was measured as described previously^{5,9}.

We obtain a rough estimate of the total repair capacity of a cell by adding the values for each of the three processes. The two clinically normal individuals thus have total repair capacities of 300 (for HESM) and 240 (for CeAr). The latter individual is the mother of one normal and five XP children; although she (like other presumed heterozygotes for excision repair¹) has normal unscheduled synthesis, her PRE level is about half normal. This finding indicates that a small loss of repair capacity may be tolerated without apparent detriment.

XP30RO has only mild to moderate clinical symptoms of XP and an intermediate repair capacity of 173, whereas variants with severe symptoms (XP4BE, XP13BE and XP7TA) have even lower repair capacities—143, 141 and 137, respectively.

It is likely that the three repair processes are not equally important in cellular DNA repair *in vivo*. Assignment of a 10-fold greater importance, however, to excision repair than to postreplication repair and photoreactivation led to predictions of similar repair capacities for normal and XP variants, in contradiction to their clinical symptoms. Similarly, assignment of 10-fold greater importance to photoreactivation or postreplication repair led to the prediction that CeAr should be XP instead of normal, or that all XP variants should show the same degree of affliction with XP respectively. It is thus possible that all three repair processes are necessary for normal repair—perhaps because of the lack of overlap of the repair processes or availability of the DNA to the repair enzymes in space or time¹⁰.

In spite of the apparent correlation of the clinical symptoms with the calculated total repair capacity, at least three variables—differential sun exposures of individuals to sun, variable shielding by pigments in the damage and differing ages on clinical examination—make the use of such a simple scheme of computation of DNA repair capacity difficult. The apparent

Table 2 Repair capacities in normal and XP cells

Cell line	Phenotype	Unscheduled* DNA synthesis (% normal)	Relative† DNA size after irradiation (% normal)	PRE Activity (% normal)	Total repair capacity
Normal					
HESM	Normal	100§	100§	100	300
CeAr‡	Normal (ref. 1)	100 (ref. 1)	100§	34	234
Variants					
XP30RO	Light/moderate XP (ref. 8)	88 (ref. 8)	29.2 (ref. 8)	56	173
XP4BE (Wo Mec)	Severe XP	100 (ref. 8)	32§	11	143
XP13BE (Pe Hay)	XP	100 (ref. 1)	32§	9	141
XP7TA	Severe XP	99 (ref. 8)	34 (ref. 8)	4	137

*Unscheduled DNA synthesis is a measure of excision repair¹.

†Relative DNA size 1 h after ultraviolet irradiation is a measure of postreplicative repair ability.

‡Parent of one normal and five XP children¹.

§Although these cell lines were not tested, the values shown are typical for normal (HESM and CeAr) and XP variant (XP13BE) cells, respectively.

correlation of DNA repair capacity with clinical signs or propensity to sunlight-induced skin malignancy—and the severity of such disease—makes it tempting to speculate that there is a direct relationship between the total DNA repair capacity and development of sunlight-induced skin cancer.

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BETSY M. SUTHERLAND
ROWENA OLIVER

Department of Molecular Biology and Biochemistry,
University of California, Irvine, California 92664

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- ¹ Robbins, J. H., Lutzner, M. A., Festoff, B. W., and Coon, H. G., *Ann. Int. Med.*, **80**, 221–284 (1974).
- ² Robbins, J. H., Levis, W. R., and Miller, A. E., *J. Invest. Dermat.*, **58**, 402–408 (1974).
- ³ Setlow, J. K., *Curr. Top. Radiat. Res.*, **2**, 195–248 (1966).
- ⁴ Sutherland, B. M., *Nature*, **248**, 109–112 (1974).
- ⁵ Sutherland, B. M., Rice, M., and Wagner, E. K., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 103–107 (1975).
- ⁶ Setlow, R. B., *Science*, **153**, 379–386 (1966).
- ⁷ Lehmann, A. R., *Life Sci.*, **15**, 2005–2016 (1975).
- ⁸ Lehmann, A. R., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 219–223 (1975).
- ⁹ Sutherland, B. M., and Chamberlin, M. J., *Analyt. Biochem.*, **53**, 168–176 (1973).
- ¹⁰ Buhl, S. N., and Regan, J. D., *Biophys. J.*, **14**, 519–527 (1974).
- ¹¹ Ibsen, K. I., and Trippet, P., *Archs Biochem. Biophys.*, **163**, 570–580 (1974).
- ¹² Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).

Rat liver microsomes catalyse covalent binding of ¹⁴C-vinyl chloride to macromolecules

THE occurrence of angiosarcoma and injury of the liver in workers occupationally exposed to vinyl chloride (VC) has focused interest on the fate of VC in the organism. Jaeger *et al.*¹ presented evidence that pretreatment of rats with phenobarbital, which induces the microsomal mixed function oxidase system of the liver, also enhances liver toxicity of VC. They suggested that VC may be transformed by this enzymic system to a reactive epoxide intermediate which has also been considered by some other authors^{2,3}. This intermediate, according to its electrophilic properties, might interact with components of the liver cell. We present here some results which further support this theory.

We incubated 1,2-¹⁴C-VC with rat liver microsomes and an NADPH-regenerating system (isocitrate and isocitric dehydrogenase). Preparation of microsomes and the incubation mixture followed standard procedures⁶. The incubation vessels were connected to an all-glass system containing the radioactive VC substrate which was diluted with atmospheric air, so that incubations could be carried out at different rates of supply of the gaseous substrate. After incubation (90 min) the total uptake of substrate during incubation was determined by measuring the total radioactivity present in the incubation mixture. Irreversible binding of VC metabolites to the microsomal protein was determined after exhaustive solvent extrac-

tions as described previously using other lipophilic substrates^{4–6}. It is known that rat liver converts VC to metabolites such as chloroethanol, chloroacetaldehyde and chloroacetic acid^{3,7}. Therefore extraction of the proteins had to remove the lipophilic VC and the water-soluble metabolites. Exhaustive extraction of the microsomal proteins was achieved using a schedule originally developed for removing metabolites of imipramine⁸. This included precipitation of proteins with ethanol and washing of the precipitate with 70% ethanol, Tris buffer, pH 7.5 (twice), 70% ethanol, absolute ethanol (twice), acetone–chloroform (4:1, twice), and finally 70% ethanol. Irreversible binding of VC metabolites to soluble proteins and nucleic acids which had been added to the microsomal incubation was estimated after adsorption of the lipophilic VC to charcoal (norit A) and subsequent dialysis of the water-soluble metabolites. After these procedures no further radioactivity could be removed from the soluble proteins or nucleic acids if these were subjected to solvent extraction (70% ethanol at –20 °C). The radioactivity which was attached to the protein remained constant during this extraction procedure.

Table 1 shows that supply of NADPH was essential to achieve covalent binding of VC metabolites to rat liver microsomes. In addition, VC metabolites were also bound to albumin when this was added to the microsomal incubation. Non-mercapto-albumin was prepared according to Carlsson and Svenson⁹. This preparation was much less efficient in trapping the reactive intermediate formed from VC by the microsomal enzymes. Concanavalin A (con A), containing no cysteine at all, does not covalently bind VC metabolites, indicating that cysteine participates in the binding reaction. The results are in agreement with the assumption of VC-epoxide being the reactive VC metabolite. Similar conclusions have been drawn for metabolic activation of vinylidene chloride⁹.

Further experiments fitted this concept. Covalent binding of VC metabolites to proteins in our system was depressed by 30% on addition of 1 mM glutathione. Addition of cytosol, which contains glutathione, had a similar effect.

The inhibitor of microsomal cytochrome P450-dependent mixed function oxidations, 1-naphthyl-4(5)-imidazole¹⁰ at a concentration of 10^{–4} M, inhibited covalent binding by 85%.

The finding that VC metabolites can also be bound to RNA (Table 1) seems to be of particular importance with regard to the mechanism of chemical carcinogenesis induced by VC. It is commonly accepted that pre-carcinogens are primarily metabolised to electrophilic ultimate carcinogens which can bind covalently to cellular macromolecules¹¹.

Additional support for the involvement of epoxide in the covalent binding reaction could be obtained from experiments using the xanthine oxidase model system which generates H₂O₂ and the O₂[–] radical¹². These incubations (1 ml) contained: 10 mg albumin, 0.8 mM hypoxanthine, 8 mU xanthine oxidase (Boehringer, Mannheim) and 0.08 mM EDTA, and were exposed to the ¹⁴C-VC-containing atmosphere for 60 min. Control incubations with albumin and 5 mM H₂O₂ showed no covalent binding of ¹⁴C-VC to the albumin, whereas the complete xanthine oxidase system, when taking up 10 nmol ¹⁴C-VC,

Table 1 Covalent binding of metabolites of ¹⁴C-VC in rat liver microsomal incubations*

	Binding to microsomal protein (nmol VC metabolites bound to 1 mg microsomal protein)	Binding to proteins and RNA added to the microsomal incubations (nmol VC metabolites bound to 10 mg macromolecular compound, as catalysed by 1 mg microsomal protein)†			
		Bovine serum albumin‡	Non- mercaptoalbumin§	Con A¶	RNA
Without NADPH-regenerating system	<0.001	<0.001	<0.001	<0.001	<0.001
With NADPH-regenerating system	0.14 ± 0.07 (mean ± s.d.; n = 9)	0.15	0.07	<0.001	0.12

* 90 min, 37 °C. Volume of incubation = 1 ml. Uptake of substrate (VC) = 10 nmol in presence of NADPH.

† Mean values of double determinations.

‡ Contains cys–SH and cys–SS–cys.

§ Contains cys–SS–cys.

¶ Contains no cys.

bound 12.6 ± 1.2 pmol (mean $\pm$ s.d.; $n = 3$) VC metabolites to the 10 mg albumin. This strongly suggests that O_2^- radicals, which are known to be involved in epoxidation by the microsomal enzyme system¹³, convert VC to a metabolite which then binds covalently to a protein-like albumin.

In accordance with our results Bartsch *et al.*^{9,14} reported that in bacterial test systems VC and also the closely related compound vinylidene chloride are only found to be mutagenic if rat liver microsomes are present to form the ultimately mutagenic metabolite. Pretreatment of the rats with phenobarbital enhances the mutation rate. This also corresponds to the work of Jaeger *et al.*¹ on liver toxicity of VC in phenobarbital-pretreated rats. Thus, the concept of a reactive metabolite of VC involved in carcinogenesis and liver toxicity gains further support.

H. KAPPUS
H. M. BOLT
A. BUCHTER
W. BOLT

*Institute of Toxicology,
University of Tübingen,
D-74 Tübingen, Wilhelmstrasse 56
and Institute of Occupational and Social Medicine,
University of Cologne,
D-5 Cologne 41, Josef-Stelzmann-Strasse 9,
West Germany*

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- ¹ Jaeger, R. J., *et al.*, *Nature*, **252**, 724–726 (1974).
- ² Hefner, R. E., Watanabe, P. G., and Gehring, P. J., *Ann. N. Y. Acad. Sci.*, **246**, 135–148 (1975).
- ³ Van Duuren, B. L., *Ann. N. Y. Acad. Sci.*, **246**, 258–267 (1975).
- ⁴ Kappus, H., Bolt, H. M., and Remmer, H., *Steroids*, **22**, 203–225 (1973).
- ⁵ Bolt, H. M., and Kappus, H., *J. Steroid Biochem.*, **5**, 179–184 (1974).
- ⁶ Kappus, H., and Remmer, H., *Biochem. Pharmacol.*, **24**, 1079–1084 (1975).
- ⁷ Radwan, Z., and Henschler, D., *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.*, **suppl.**, **287**, R 100 (1975).
- ⁸ Carlsson, J., and Svenson, A., *FEBS Lett.*, **42**, 183–186 (1974).
- ⁹ Bartsch, H., Malaveille, C., Montesano, R., and Tomatis, L., *Nature*, **255**, 641–643 (1975).
- ¹⁰ Wilkinson, C. F., Hetnarski, K., and Hicks, L. J., *Pesticide Biochem. Physiol.*, **4**, 299–312 (1974).
- ¹¹ Miller, J. A., *Cancer Res.*, **30**, 559–576 (1970).
- ¹² Bors, W., Saran, M., Lengfelder, E., Spötl, R., and Michel, C., *Curr. Top. Radiat. Res.*, **9**, 247–309 (1974).
- ¹³ King, T. E., Mason, H. S., and Morrison, M., *Oxidases and Related Redox Systems*, 2 (University Park Press, Baltimore, Maryland, 1973).
- ¹⁴ Bartsch, H., Malaveille, C., and Montesano, R., *Int. J. Cancer*, **15**, 429–437 (1975).

A genetic basis for resistance to enteric disease caused by *E. coli*

THE K88 antigen on the surface strains of *E. coli* which cause neonatal diarrhoea in piglets^{1,2} is an essential virulence determinant^{3,4} because its adhesive properties enable K88-positive strains to attach to piglet intestinal mucosa^{4–7} and establish in the small intestine⁸. These bacteria multiply to reach abnormally large numbers in the gut⁶ and synthesise enterotoxins that cause diarrhoea and death of infected piglets^{9,10}. K88-positive bacteria, however, do not attach to intestinal tissue from all piglets, and at least two pig phenotypes occur^{6,7}, which we have designated 'adhesive' (signifying that K88-positive bacteria attach to their brush borders) or 'non-adhesive' (K88-positive bacteria do not attach to their brush borders). Phenotypes are the products of two alleles at a single locus which are inherited in a simple Mendelian manner⁷, and the phenotypes of progeny from selected matings support the conclusion that the product of the 'adhesive' allele is dominant over the product of the 'non-adhesive' allele (R. A. G., R. S. and J. M. R., unpublished).

Thus, it seems reasonable to postulate that K88-positive bacteria colonise the gut of 'adhesive' pigs more readily than the gut of 'non-adhesive' pigs, and that 'adhesive' animals are more likely to be susceptible to neonatal diarrhoea caused by these strains. To investigate this possibility, five litters comprising 47 piglets were removed from their dams 2 d after birth

and fed on cow's milk¹¹. Two days later, 1 ml of a suspension containing 10^{10} viable organisms of a virulent K88-positive strain W1 (0149 : K91 (B), K88ac(L); H10) of *E. coli* was given to each piglet by stomach tube. Clinical observations were made daily and severely diseased piglets either died or were killed *in extremis* within 4 d of challenge; healthy surviving piglets were killed 5–7 d after challenge. All piglets were necropsied and viable bacterial counts were made on the contents of the intestine. Brush borders were prepared from the small intestine of each piglet and its phenotype was determined⁶.

Twenty-three piglets from the five litters were phenotyped as 'adhesive' and 21 of these piglets died or were killed with clinical signs of neonatal diarrhoea. At necropsy, strain W1 was present in large numbers throughout the gut and was generally dominant in the anterior small intestine. The two 'adhesive' piglets which survived showed no clinical signs of disease and did not excrete strain W1 after challenge.

Twenty-four piglets from the five litters were phenotyped as 'non-adhesive' and 23 survived challenge. These piglets showed no clinical signs of neonatal diarrhoea and did not excrete strain W1. One 'non-adhesive' piglet died 72 h after challenge with mild signs of neonatal diarrhoea but excreted only small numbers of strain W1.

The mean $\log_{10}$ viable counts of *E. coli* in the anterior small intestine of the 23 healthy 'non-adhesive' survivors was 5.8; strain W1 ($\log_{10}$ 5.3) was present in only one of these animals. In contrast, the mean count of strain W1 was $\log_{10}$ 8.2 in 15 diseased 'adhesive' piglets that were killed, and $\log_{10}$ 9.7 in six diseased 'adhesive' pigs that died. In the two healthy 'adhesive' pigs which survived, strain W1 was not detected in the intestine, and in the diseased 'non-adhesive' piglet which died, strain W1 was present in the anterior small intestine ($\log_{10}$ 7.6) but was outnumbered almost 100-fold by other *E. coli*.

These results indicate that the 'adhesive/non-adhesive' phenotype determines whether the K88-positive enteropathogenic strain establishes in the gut and proliferates to cause clinical disease. In the 'adhesive' animals, strain W1 attaches and multiplies to reach large numbers in the small intestine, whereas in the 'non-adhesive' animals, strain W1 is unable to attach and rapidly disappears from the gut. The survival of two 'adhesive' piglets in which strain W1 failed to establish demonstrates, however, that additional factors may prevent attachment and proliferation of K88-positive bacteria in the gut. The death of one 'non-adhesive' piglet with strain W1 in the small intestine indicates that attachment by K88 is not always essential for the pathogenic strain to establish in the gut.

The complex nature of the *in vivo* situation has been emphasised in an experiment in which the expected susceptibility of sucking 'adhesive' piglets experimentally infected at birth was not expressed; these piglets received colostrum from the dam that contained K88 antibodies which are known to be associated with passive protection of piglets against challenge with strain W1^{12–14}. It seems reasonable to suggest that these 'adhesive' piglets were susceptible to infection but passive protection conferred by the K88 antibodies in colostrum and milk prevented the enteropathogenic strain from attaching and multiplying to reach high numbers in the gut.

These results have a number of interesting implications. It seems that the 'non-adhesive' phenotype primarily determines whether an animal will be resistant to infection with K88-positive strains of *E. coli*; however, if 'adhesive' animals receive protective antibodies in mammary secretions, then they may seem to be 'resistant' to infection. Although the nature of the product of the 'adhesive' allele is unknown, study of the K88 antigen has provided, as far as we are aware, the first example of a genetic basis for resistance to enteric disease. As a result, it may be possible to select breeding pigs that have the 'non-adhesive' gene and thus produce progeny resistant to neonatal diarrhoea caused by K88-positive *E. coli*.

In addition to this practical application, it is becoming clear that adhesion to tissue surfaces may be an important mechanism of pathogenicity in several diseases including human bacterial

infections caused by *Vibrio cholerae*¹⁵, and streptococci¹⁶. If the principles that we have demonstrated for K88-positive *E. coli* infection^{4,6,7,12-14} apply more generally, then the outcome of such infections may be determined by the virulence determinants of the microorganism, the phenotype of the host and the immune response of the host or its dam. An understanding of the genetic susceptibility of the host may, therefore, be an essential prerequisite for the interpretation and evaluation of infection experiments with those pathogens which become attached to cell surfaces.

J. M. RUTTER
M. R. BURROWS
R. SELLWOOD
R. A. GIBBONS

Institute for Research on Animal Diseases,
Compton, Newbury, Berkshire RG16 0NN, UK

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- ¹ Orskov, I., Orskov, F., Sojka, W. J., and Wittig, W., *Acta path. microbiol. Scand.*, **62**, 439-447 (1964).
- ² Sojka, W. J., *Vet. Bull.*, **41**, 509-522 (1971).
- ³ Smith, H. W., and Linggood, M. A., *J. med. Microbiol.*, **4**, 467-480 (1971).
- ⁴ Jones, G. W., and Rutter, J. M., *Infect. Immun.*, **6**, 918-927 (1972).
- ⁵ Wilson, M. R., and Hohmann, A. W., *Infect. Immun.*, **10**, 776-782 (1974).
- ⁶ Sellwood, R., Gibbons, R. A., Jones, G. W., and Rutter, J. M., *Vet. Rec.*, **95**, 574 (1974).
- ⁷ Sellwood, R., Gibbons, R. A., Jones, G. W., and Rutter, J. M., *J. med. Microbiol.* (in the press).
- ⁸ Smith, H. W., and Jones, J. E. T., *J. Path. Bact.*, **86**, 387-412 (1963).
- ⁹ Smith, H. W., and Halls, S., *J. Path. Bact.*, **93**, 531-543 (1967).
- ¹⁰ Smith, H. W., and Gyles, C. L., *J. med. Microbiol.*, **3**, 387-401 (1970).
- ¹¹ Chandler, R. L., Derbyshire, J. B., and Smith, K., *Res. vet. Sci.*, **10**, 435-439 (1969).
- ¹² Rutter, J. M., and Jones, G. W., *Nature*, **242**, 531-532 (1973).
- ¹³ Rutter, J. M., *Vet. Rec.*, **93**, 165 (1973).
- ¹⁴ Jones, G. W., and Rutter, J. M., *Am. J. clin. Nutr.*, **27**, 1441-1449 (1974).
- ¹⁵ Freter, R., *Infect. Immun.*, **6**, 134-141 (1972).
- ¹⁶ Ellen, R. P., and Gibbons, R. J., *Infect. Immun.*, **5**, 826-830 (1972).

Cadmium resistance and content of cadmium-binding protein in cultured human cells

INDUCTION of hepatic metallothionein (a cadmium-binding protein) is considered to be a protective mechanism in mammals against the toxic cadmium ion. Pretreatment of rats with low doses of cadmium induces the synthesis of metallothionein and also protects against subsequent exposure to an otherwise lethal dose¹. But the role of metallothionein in cadmium resistance is not clear². Restriction of food intake in the rat increases the level of metallothionein³, but does not alter the LD₅₀ of cadmium². Also the synthesis of metallothionein seems to continue for a longer time than the protection against cadmium in both rats and mice^{1,2}. Lucis, Shaikh and Embil⁴ found uptake of Cd in cultured human embryonic fibroblasts, HeLa cells and monkey kidney epithelial cells after 8 d of incubation with Cd. At that time the cells also contained a Cd-binding protein. The authors did not, however, describe growth of the cells in the presence of Cd or development of cell strains resistant to Cd. Webb and Daniel⁵ recently described the synthesis of a Cd-binding protein in cultures of cells derived from the cortex of the adult pig kidney.

We describe here the development of Cd resistance and the relationship between cadmium resistance and cadmium-binding protein (CdBP) levels in cultured human skin epithelial cells.

Human skin epithelial cells (HE cells) NCTC strain 2544 were obtained from American Type Culture collection, Rockville, Maryland and grown in Falcon plastic tissue culture flasks (75 cm²) in Dulbecco's modified Eagle's medium supplemented with foetal calf serum and horse serum as described in ref. 6. Medium was changed every 3 d. Cadmium (20 µM) added to the medium had toxic effects; after 2-3 d the cells altered shape, about 90% of them died and the growth of those remaining ceased. After a lag period of about a week, the cells retained their growth. The Cd concentration was kept at 20 µM and after 5-6 weeks the cells had regained a normal appearance and an almost normal growth rate, that is, a generation time of about 24 h. At that time the Cd concentration was increased

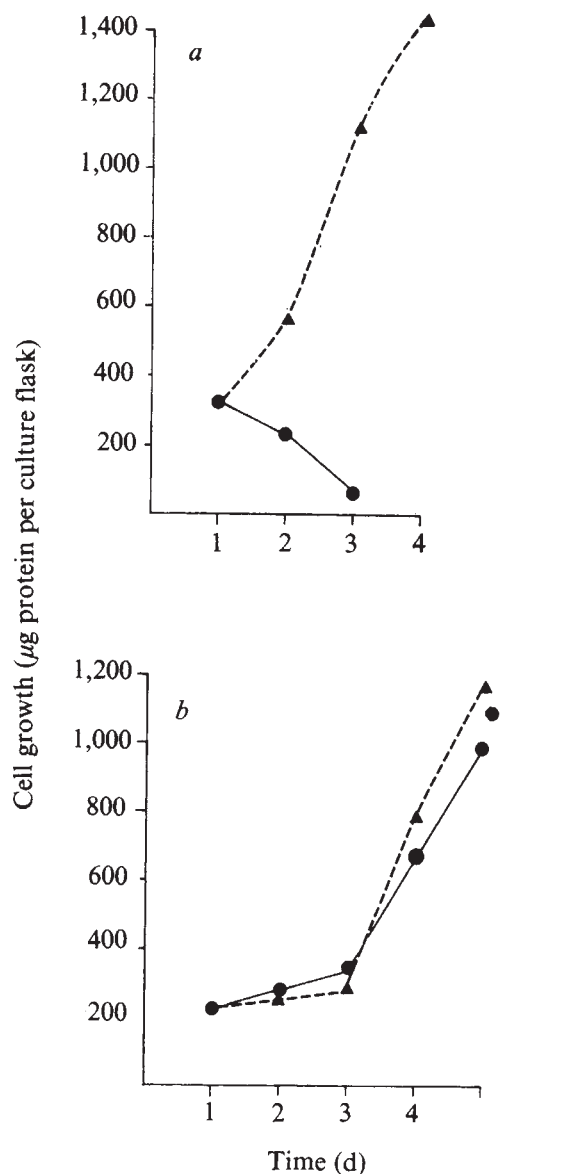


Fig. 1 Growth of human epithelial cells not made resistant to cadmium (a) and human epithelial cells made resistant to 100 µM Cd in the growth medium (b). Both cell lines were grown in medium without Cd added (▲) and with 100 µM Cd added (●).

to 40 µM. The cells again altered morphology, most of them died and the growth ceased temporarily, but in the course of some weeks, the cells regained normal growth. By increasing the Cd concentration in the medium about every 6 weeks, the cells were made resistant to 100 µM Cd in about 30 weeks. After 30 weeks the resistant cells had about the same growth rate in the presence of 100 µM Cd as in Cd-free medium (Fig. 1). This concentration of Cd would kill HE cells not previously exposed to Cd. The resistant cells could be frozen in liquid nitrogen for several months without losing the resistance towards Cd. The cells could also be grown for at least 4 weeks without Cd in the medium without losing Cd resistance. These Cd-resistant HE cells have a generation time of approximately 24 h (like the parent cell line) and the resistance is thus persistent for at least 20 generations without any Cd added to the growth medium.

Growth medium and cells were tested for low molecular weight CdBP by addition of ¹⁰⁹Cd, a gamma emitter, to the cell cultures. To get a maximum uptake of labelled Cd, cultures of resistant cells were grown for at least 3 d in a Cd-free medium before the addition of ¹⁰⁹Cd. Labelled Cd (final concentration 1.3 µM) was then added to the growth medium. After growth

for 24 h in medium containing ^{109}Cd , the medium was collected, the cells were washed twice with 5 ml prewarmed (37°C) medium without Cd, lysed in 10 ml distilled water, and frozen and thawed twice. The cell lysate was centrifuged at $2,000g$ for 10 min and the supernatant chromatographed on a Sephadex G-75 column (K 16/100) 1.6×79 cm in Tris-HCl buffer 0.001 M , pH 8.6.

The column was calibrated with bovine albumin, trypsin inhibitor soyabean protein and cytochrome *c*. The result of gel filtration of the lysate of HE cells resistant to $100\text{ }\mu\text{M}$ Cd grown in the presence of ^{109}Cd is shown in Fig. 2. The elution volume for peak 2 was the same as for cytochrome *c* and corresponds to a molecular weight of about 12,000.

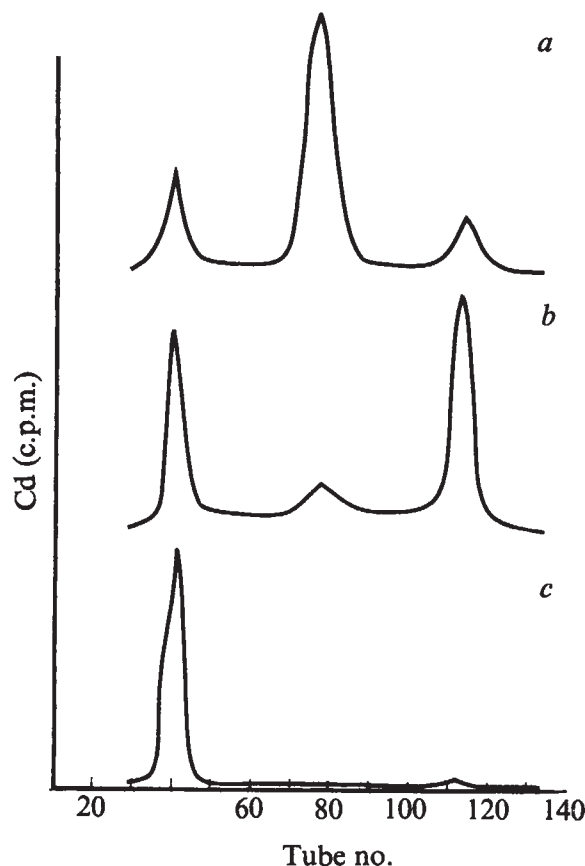


Fig. 2 Gel filtration (Sephadex G-75) of lysate of: *a*, human skin epithelial cells made resistant to $100\text{ }\mu\text{M}$ Cd; *b*, cells not resistant to Cd; *c*, growth medium (Dulbecco's modification of Eagles' medium supplemented with 17.5% serum and with $1.3\text{ }\mu\text{M}$ ^{109}Cd). Tube 40 represents the exclusion volume (molecular weight about 70,000), tube 80 corresponds to a molecular weight of about 12,000; tube 120 is the total volume of the column and corresponds to a molecular weight of less than 1,000–3,000.

Figure 2 also shows the result of gel filtration of lysate of HE cells not resistant to Cd also grown for 24 h in the presence of $1.3\text{ }\mu\text{M}$ ^{109}Cd . No peaks corresponding to CdBP of molecular weight about 12,000 could be detected. Growth medium likewise did not contain any CdBP with a molecular weight of 12,000. A Cd peak was demonstrated corresponding to a protein eluted with the void volume, which means that its molecular weight is above 70,000. Both types of cell had a Cd peak corresponding to the total volume of the column, that is Cd conjugated to some compound with a molecular weight of less than 1,000–3,000. HE cells resistant to $100\text{ }\mu\text{M}$ Cd, but grown without any Cd added to the medium for 4 weeks, also contain a Cd-binding macromolecule with a molecular weight of 12,000. After about 20 generations in Cd-free medium these cells were still resistant to $100\text{ }\mu\text{M}$ Cd and they also still had CdBP with a molecular weight of 12,000. We do not know,

however, whether minute amounts of Cd were still present in the cells at that time. In some experiments Cd isotope was added to the medium and the cell lysate after collection of the cells. The results were essentially the same; CdBP of molecular weight about 12,000 could be demonstrated in lysates from Cd-resistant cells, but not in the growth medium or in HE cells not exposed to Cd.

In these experiments we have thus found a good correlation between Cd resistance and the appearance of CdBP. Since our epithelial cells have been derived from human skin, these experiments show that CdBP may be produced in organs other than liver and kidney. Two different cadmium-binding proteins (metallothioneins) have been purified from human livers by Bühler and Kägi⁶. They determined the molecular weights to be about 6,100 for the peptide moiety. Nordberg *et al.*⁸ isolated and described metallothionein from rabbit with a molecular weight of about 6,000. Weser *et al.*⁹ found, however, a molecular weight of about 12,000 for rat and chicken. They discuss whether their protein might be a dimer of the rabbit protein isolated by Nordberg *et al.*⁸. The protein which seems to develop parallel to the resistance to cadmium in the cells used by us may constitute a dimer of the protein described by Bühler and Kägi⁷. Kägi and Vallee¹⁰ previously described an equine protein containing cadmium and zinc with a molecular weight of about 10,000, but they do not discuss the possibility of dimer generation.

We believe that these cells are well suited for the study of induction of cadmium-binding proteins and their biological role, including the possible correlation between Cd resistance and content of Cd-binding proteins.

HANS ERIK RUGSTAD

*Institute of Pharmacology,
University of Oslo, Blindern*

TOR NORSETH

*Institute of Occupational Health,
Gydas vei 8,
Oslo 3, Norway*

Received June 23; accepted July 28, 1975.

- Yoshikawa, H., *Ind. Hlth.*, **8**, 184–191 (1970).
- Webb, M., *Ninth FEBS Meeting*, 25–30 August, Budapest (1974).
- Brenner, I., Davies, N. T., and Mills, C. F., *Biochem. Soc. Trans.*, **1**, no. 4, 982–989 (1973).
- Lucis, O. J., Shaikh, Z. A., and Embil, Jr., J. A., *Experientia*, **26**, 1109–1110 (1970).
- Webb, M., and Daniel, M., *Chem. Biol. Interactions*, **10**, 269–276 (1975).
- Rugstad, H. E., and Dybing, E., *Eur. J. clin. Invest.*, **5**, 133–137 (1975).
- Bühler, R. H. O., and Kägi, J. H. R., *FEBS Lett.*, **39**, 229–234 (1974).
- Nordberg, G. F., Nordberg, M., Piscator, M., and Vesterberg, O., *Biochem. J.*, **126**, 491–498 (1972).
- Weser, U., *et al.*, *Eur. J. Biochem.*, **39**, 127–140 (1973).
- Kägi, J. H. R., and Vallee, B. L., *J. biol. Chem.*, **236**, 2435–2442 (1961).

Ricin resistance in baby hamster kidney cells

LITTLE is understood about the function of the complex oligo-saccharide side chains present in cell membrane glycoproteins. One approach to this problem has been to select from cultured mammalian cells variants deficient in cell surface binding sites for certain plant lectins, since in these cells some part of the membrane glycoproteins' carbohydrate moieties should be missing or altered. Such variants have been isolated from Chinese hamster ovary (CHO) cells using ricin¹ (from *Ricinus communis* seeds) and phytohaemagglutinin² (from *Phaseolus vulgaris*), cytotoxic lectins which bind to membrane glycoprotein galactose and N-acetylgalactosamine groups^{3–5}.

We describe here 22 clones of the baby hamster kidney cell line, BHK 21 C13 (ref. 6) resistant to ricin, several of which seem to be deficient in cell surface ricin binding sites.

In our studies BHK cells (approximately 2×10^5) growing in Glasgow modified minimal essential medium (MEM) plus 10% foetal bovine serum (FBS) at 33°C on 60-mm Falcon plastic dishes were treated with methyl-N-nitro-N-nitrosoguanidine

($0.5 \mu\text{g ml}^{-1}$) for 1 d. Surviving cells (about 28% of the total) were grown for a further 4 d to establish mutant phenotypes, trypsinised and replated at 5×10^5 cells per dish in MEM plus 2% FBS. Since FBS contains ricin-binding glycoproteins the lower concentration was used in the selection procedure. The cells were grown to approximately 3×10^6 per dish at 39°C , ricin ($0.2 \mu\text{g}$ or $1 \mu\text{g ml}^{-1}$) was added and incubation continued for 7–10 d in the presence of ricin with the medium changed every 3 d. In some experiments the ricin concentration was increased from $0.2 \mu\text{g}$ to $1 \mu\text{g ml}^{-1}$ after the first 3 d. Many

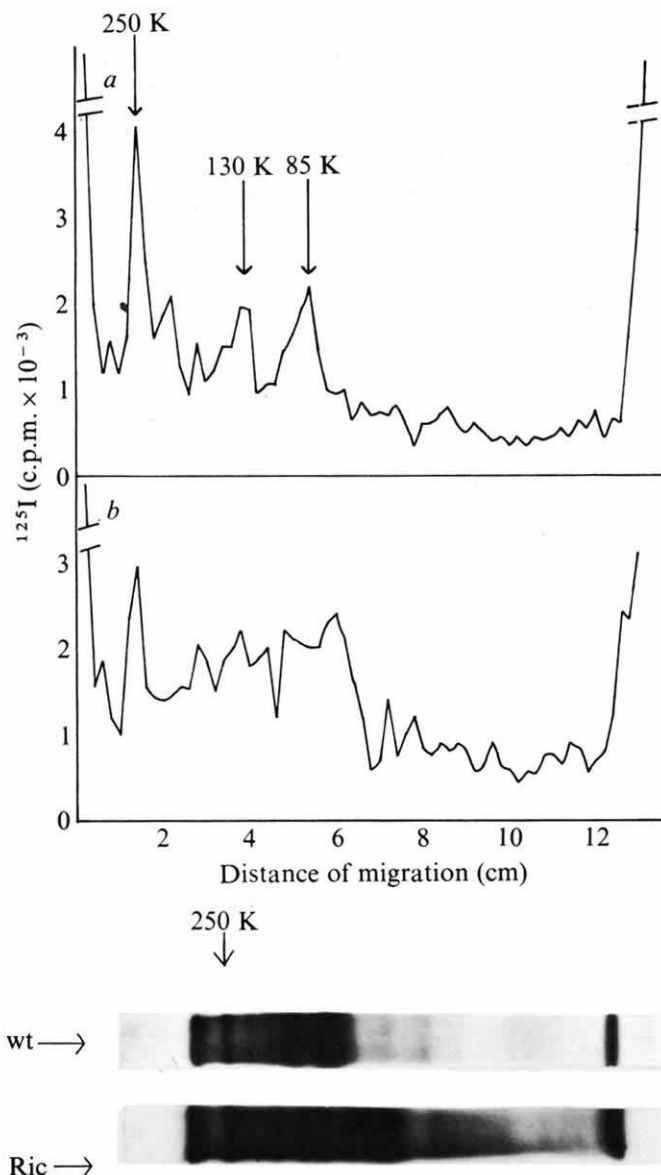


Fig. 1 Lactoperoxidase-catalysed surface iodination of baby hamster kidney (BHK) cells. Intact cells of normal BHK cells and the ricin resistant variant Ric^R14 were iodinated with ^{125}I by standard lactoperoxidase labelling techniques. The iodinated cells were dissolved in hot sodium dodecyl sulphate plus 2-mercaptoethanol and examined by slab gel polyacrylamide gel electrophoresis. The radioiodinated bands represent surface components accessible to lactoperoxidase, an enzyme that does not penetrate the plasma membrane of intact cells to label cytoplasmic proteins or glycoproteins. The bands were detected (top panel) by autoradiography or by (lower panel) cutting up the gel into 2-mm slices and counting in a Wallac gamma spectrometer. Approximate molecular weights of proteins were estimated by reference to the mobilities of reovirus structural proteins¹⁵. *a*, Normal cells at confluence; *b*, ricin-resistant cells Ric^R14 at high cell density (confluence). Note the appearance of a major iodinated band of apparent molecular weight 250,000 in the normal and Ric^R14 cells at confluence. Note the enhanced mobility of the other iodinated bands present in Ric^R14 cells relative to the corresponding iodinated components of the normal BHK cells.

Table 1 Selection, resistance and ricin binding sites of ricin-resistant BHK cell clones

Cells	Ricin concentration for selection	Relative plating* efficiency (±ricin)%	Relative binding† of ^{125}I -ricin
Normal	—	1–2	1.0
Ric ^R 1	$0.2 \mu\text{g ml}^{-1}$	35	1.12
2	$0.2 \mu\text{g ml}^{-1}$	25	1.12
3	$0.2 \mu\text{g ml}^{-1}$	2.5	ND
4	$0.2 \mu\text{g ml}^{-1}$	ND	ND
5	$0.2 \mu\text{g ml}^{-1}$	17	ND
6	$1 \mu\text{g ml}^{-1}$	60	0.15
7	$1 \mu\text{g ml}^{-1}$	83	0.43
8	$1 \mu\text{g ml}^{-1}$	91	ND
9	$1 \mu\text{g ml}^{-1}$	50	0.12
10	$1 \mu\text{g ml}^{-1}$	40	0.13
11	$1 \mu\text{g ml}^{-1}$	83	ND
12	$1 \mu\text{g ml}^{-1}$	43	1.36
13	$1 \mu\text{g ml}^{-1}$	75	ND
14	$0.2 \mu\text{g ml}^{-1}$	43	0.12
15	then $1.0 \mu\text{g ml}^{-1}$	107	0.14
16	$1.0 \mu\text{g ml}^{-1}$	50	1.05
17	$1.0 \mu\text{g ml}^{-1}$	35	0.27
18	$1.0 \mu\text{g ml}^{-1}$	70	0.47
19	$1.0 \mu\text{g ml}^{-1}$	70	1.97
20	$1.0 \mu\text{g ml}^{-1}$	51	0.19
21	$1.0 \mu\text{g ml}^{-1}$	83	0.10
22	$0.2 \mu\text{g ml}^{-1}$	40	1.5
	then $0.5 \mu\text{g ml}^{-1}$		

* Trypsinised cells in Ham's F10 medium plus 10% foetal calf serum were plated on 60-mm Falcon dishes at approximately 1,000 cells per dish. After 1–2 h at 39°C ricin was added at $0.1 \mu\text{g ml}^{-1}$ final concentration and after further incubation for 7–10 d, cell colonies were stained with 1% gentian violet in 20% ethanol and counted. The relative plating efficiency is the number of colonies developed in the presence of ricin compared with the number of colonies developed in the absence of ricin from equal numbers of cells.

† Cells growing on 35-mm diameter plastic dishes were rinsed with warm phosphate-buffered saline. Serial dilutions of a solution of lactoperoxidase-catalysed, ^{125}I -labelled lectin (usually at least 10^7 c.p.m. mg^{-1} of protein) were added (each 1 ml). After incubation at room temperature for 60 min the cells were rinsed several times to remove unbound lectin, dissolved in alkali and assayed for cell protein and ^{125}I radioactivity. Binding curves were treated as Scatchard plots to obtain the total number of binding sites per cell. Values are expressed relative to normal BHK cells. The number of ricin binding sites for normal BHK cells is 5.6×10^6 per cell.

cells detached from the plastic surface and were removed during the changes of medium. Colonies surviving at a frequency of approximately 10^{-6} in ricin ($1 \mu\text{g ml}^{-1}$) were removed by trypsinisation under sterile glass cloning cylinders, transferred to Falcon dishes containing ricin-free fresh Ham's F10 medium containing 10% FBS, and grown to high densities to establish cell lines which thereafter were cultured in MEM plus 10% FBS. No surviving colonies were obtained from non-mutagenised cells treated with ricin at $1 \mu\text{g ml}^{-1}$.

All of our clones except one (Ric^R3) retain a substantial degree of their resistance when cultured in the absence of ricin (Table 1), even for 1 yr, and therefore exhibit stable heritable alterations. Resistance to ricin is expressed equally at both 33°C and 39°C , and thus no clone exhibits temperature-sensitive resistance, which we had hoped our selection method would provide. Of the 22 resistant clones several of those tested, for example Ric^R1, 2, 12, 16, 19, bind about as much or more ricin as do normal sensitive BHK cells (Table 1). It is likely that in these cells resistance is mediated by either a lack of endocytosis of bound lectin⁷ or an inability of the lectin once in the cytoplasm to affect protein synthesis^{8,9}. Other resistant clones, however, are deficient (Table 1) in ricin binding sites, for example, Ric^R9, 14, 15, 21.

Ricin-resistant clones deficient in lectin binding sites are probably defective in biosynthetic steps involved in their assembly. An example of such a step is the addition of N-acetylglucosaminyl residues to the internal mannose core of growing oligosaccharide side chains of surface membrane glycoproteins terminating in the sequence sialyl-galactosyl-N-acetylgluco-

saminyl-mannose¹⁰. Clones of CHO cells deficient in binding sites for phytohaemagglutinin and ricin, respectively, are deficient in a β -N-acetylglucosaminyl transferase^{11,12}, and presumably cannot transfer N-acetylglucosamine or the penultimate galactose to those glycoprotein oligosaccharides necessary for lectin binding. Enzymatic analysis of cell extracts of some of our ricin-resistant BHK clones which are deficient in binding sites, that is Ric^R14, 15, 18 and 21, has indicated that Ric^R14, but not the others, has less than 10% of normal β -N-acetylglucosaminyl transferase activity when neuraminidase, β -galactosidase and β -N-acetylglucosaminidase-treated α_1 -acid glycoprotein is used as acceptor¹³. The activities of sialyl and galactosyl transferases in all these clones, including Ric^R14, assayed using the appropriate derivatives of the α_1 -acid glycoprotein are similar to normal BHK cells. It is possible, of course, that small differences in specific glycosyl transferases, for example, a galactosyl transferase, cannot be detected or that α_1 -acid glycoprotein derivatives are not suitable acceptors for all transferases, or simply that the biosynthetic block lies elsewhere, for example, N-acetylgalactosaminyl transferase.

Additional evidence that our ricin-resistant clones deficient in binding sites are blocked at some stage of carbohydrate chain assembly comes from surface labelling. Several surface components of normal BHK cells are labelled by ¹²⁵I-iodination catalysed by lactoperoxidase (Fig. 1). In clones Ric^R14 (Fig. 1), Ric^R7, Ric^R15 and possibly Ric^R21 (not shown) the radioactive bands move more rapidly during polyacrylamide gel electrophoresis than the radioactive bands of normal cells, which is consistent with the surface glycoproteins of these clones being less well glycosylated. In contrast, the surface glycoproteins of Ric^R16 and Ric^R19, resistant clones not deficient in ricin binding sites (Table 1), show no increase in migration on polyacrylamide gels (R.N., unpublished). It is interesting that the large iodinated glycoprotein of apparent molecular weight 250,000, described by others in various cells¹⁴, is present in normal BHK cells and in the Ric^R14 clone (Fig. 1) when the cells are at high density, and that the mobility of this large glycoprotein apparently does not change in the ricin-resistant clone. Either this large glycoprotein does not bind ricin and is unaffected by the glycosylation defect, or the carbohydrate is a minor part of the whole molecule and decreased glycosylation is not detected by our method.

Further evidence for premature termination of glycoprotein oligosaccharides in our clones deficient in ricin binding sites comes from binding studies with the lectin concanavalin A (con A) with specificity for mannosides. In clones Ric^R14, 15 and 21 the number of ¹²⁵I-con A binding sites is increased 1.5–3.0-fold, suggesting increased exposure of terminal mannose groups.

We conclude therefore that stable alterations in surface glycoprotein oligosaccharides can be selected for in BHK cells. We have isolated clones which bind ricin poorly due presumably to a loss of surface galactose or N-acetylgalactosamine, and in one case (Ric^R14) we have demonstrated an enzyme deficiency in a β -N-acetylglucosaminyl transferase which could lead to such a loss. The alterations to surface glycoprotein oligosaccharides seem, however, to have negligible effects on the normal growth processes of our variants. Nevertheless, the availability of stable variants of BHK cells showing altered carbohydrate structure will be useful in studies of the role of surface carbohydrates in intercellular properties such as adhesion—several of our variant cells, particularly Ric^R14, show weaker adhesion to plastic culture surfaces—and metabolic cooperation.

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A. MEAGER
A. UNGKITCHANUKIT
R. NAIRN
R. C. HUGHES

National Institute for Medical Research,
Mill Hill, London NW7 1AA, UK

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- ¹ Gottlieb, C., Skinner, A. M., and Kornfeld, S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1078–1082 (1974).
- ² Stanley, P., Caillibot, V., and Siminovich, L., *Somatic Cell Genet.*, **1**, 1 (1975).
- ³ Nicolson, G. L., and Blaustein, J., *Biochim. biophys. Acta*, **266**, 543–547 (1972).
- ⁴ Nicolson, G. L., Blaustein, J., and Etzler, M., *Biochemistry*, **13**, 196–204 (1974).
- ⁵ Irimura, T., Kawaguchi, T., Terao, T., and Osawa, T., *Carbohydrate Res.*, **39**, 317–327 (1975).
- ⁶ Stoker, M., and MacPherson, I., *Nature*, **203**, 1355–1357 (1964).
- ⁷ Nicolson, G. L., *Nature*, **228**, 628–630 (1974).
- ⁸ Olsson, S., and Pihl, A., *Biochemistry*, **12**, 3121–3126 (1973).
- ⁹ Carrasco, L., Fernandez-Puentes, C., and Vazquez, D., *Eur. J. Biochem.*, **54**, 499–503 (1975).
- ¹⁰ Hughes, R. C., *Prog. Biophys. molec. Biol.*, **26**, 189–268 (1973).
- ¹¹ Juliano, R. L., and Stanley, P., *Biochim. biophys. Acta*, **389**, 401–406 (1975).
- ¹² Gottlieb, C., Baenziger, J., and Kornfeld, S., *J. biol. Chem.*, **250**, 3303–3309 (1975).
- ¹³ Schachter, H., et al., *J. biol. Chem.*, **245**, 1090–1100 (1970).
- ¹⁴ Hynes, R. O., *Cell*, **1**, 147–158 (1974).
- ¹⁵ Smith, R. E., Zweerink, H. F., and Joklik, W. K., *Virology*, **39**, 791–800 (1969).

Liquid-crystalline characteristics of the thick filament lattice of striated muscle

THE lattice of thick filaments (A-band lattice) in striated muscle is similar in many respects to smectic liquid-crystalline structures in which macromolecules are arranged parallel to and equidistant from each other forming lattice planes^{1,2}. Like other liquid-crystalline systems, this lattice may exist in two characteristic conditions which are determined either by a simple balance between electrical forces^{3–5,7} or subject to an additional volume-limiting force^{6–8}. Some mathematical analyses^{2,4–6,9–12} agree with the experimental results obtained

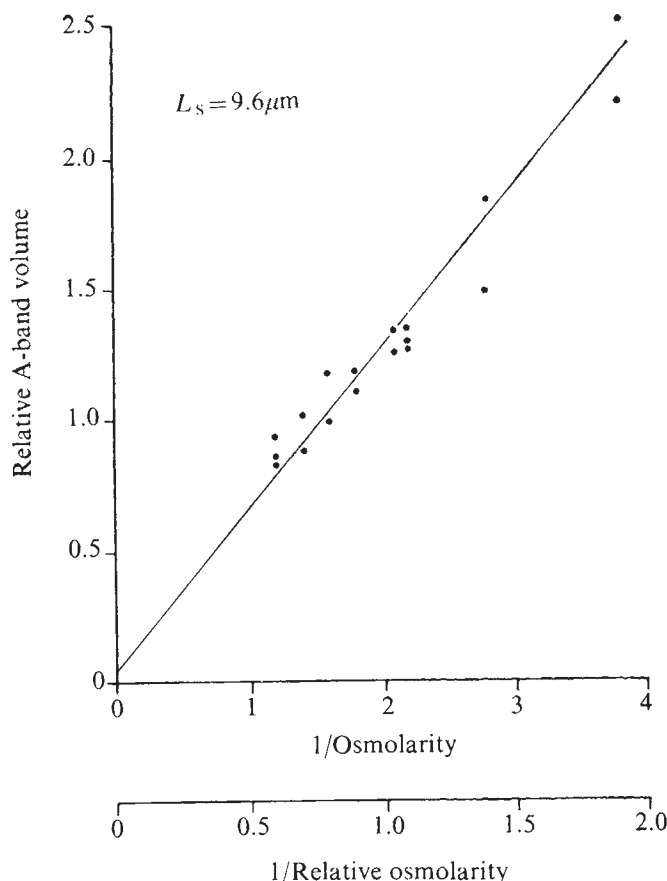


Fig. 1 A-band volume of muscle as a function of osmolarity. The relative volume of the A-band in living crayfish muscle fibres is plotted against the reciprocal osmolarity of sodium chloride media. The A-band volume across the whole fibre was determined from the optically measured diameter of the fibre at a constant sarcomere length and the dimensions of the thick filaments (4.4 μ m). The osmolarity was varied by adjusting the NaCl concentration of the normal medium (NaCl, 200 mmol l⁻¹; CaCl₂, 13.5 mmol l⁻¹; KCl, 5 mmol l⁻¹; Tris, 5 mmol l⁻¹, buffered to pH 7.4).

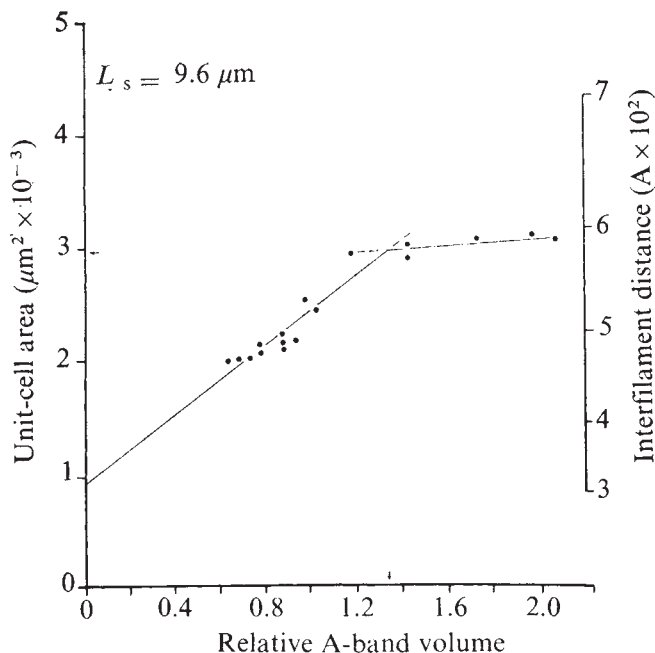


Fig. 2 Unit-cell area of the thick filament lattice of intact fibres as a function of the A-band volume. The unit-cell area and interfilament distance of the thick filament lattice of crayfish muscle at constant sarcomere length is plotted against the relative A-band volume as determined by the osmolarity of the sodium chloride media (compare Fig. 1). The close approximation of the data to a straight line (fitted by the method of least squares, $r = 0.92$) over a portion of the range indicates a region in which the lattice exhibits the Boyle-van't Hoff pressure-volume relationship of the fibre. In this region the lattice behaviour is similar to that of the volume-constrained liquid-crystal. The ordinate intercept indicates an osmotic dead space. The break in the line at an A-band volume of 135% of the control demonstrates that the unit-cell area of the thick filament lattice no longer follows the A-band volume, indicating that the lattice changes from a volume-constrained to an electrically balanced condition when the thick filament separation reaches approximately 582 Å at a sarcomere length of 9.6 μm .

from glycerinated muscle³ and skinned fibres¹³⁻¹⁵, but are only applicable to the electrically balanced liquid-crystalline condition. The behaviour of the thick filament lattice in intact living muscle cannot be explained exclusively in terms of a balance between Van der Waals' and electrostatic force⁵ because the Donnan-osmotic steady state across the sarcolemma determines the net water flux and thereby controls the fibre and lattice volumes. Cross bridges or M-line bridges cannot be evoked as a mechanism for maintaining the lattice regularity because the array persists in EGTA-relaxed skinned fibres in muscles having no M-line structure¹¹.

The volume of a single muscle fibre of crayfish (*Orconectes*), and in particular the A-band volume, is a function of the osmolarity of the bathing medium. This is evident from Fig. 1, in which the relative A-band volume across the fibre (optically determined fibre diameter times thick filament length) is plotted against the reciprocal of the osmolarity of the medium. The approximation of the data to a straight line (fitted by the method of least squares, $r = 0.97$) indicates compliance with the Boyle-van't Hoff pressure-volume relationship. In physiological conditions with the usual complement of myofilaments, the fibre volume is less than that necessary for the attainment of a balance between Van der Waals' attractive and Coulombic repulsive forces. The unit-cell volume of the sarcomere (the unit-cell volume of the thick filament lattice extended to the Z-lines) and the thick filament separation of the intact fibre also follows the Boyle-van't Hoff relationship exhibited by the whole fibre¹⁵. Figure 2 shows that the thick filament spacing is a function of A-band volume.

The unit-cell area of the A-band lattice (determined from the 1, 0 X-ray diffraction reflections of single fibres at constant sarcomere length) is plotted against the A-band volume of the whole fibre (determined by the osmolarity of the medium and measured optically). The linear fit ($r = 0.92$) of the data signifies agreement with the Boyle-van't Hoff equation and the ordinate intercept is indicative of an osmotic dead space¹⁵. These data are consistent with volume-constrained liquid-crystalline behaviour in which elongate particles comprising the lattice can be confined to a volume smaller than that which is necessary for an electrical balance¹⁶. Thus, interaxial distance between the particles (d) is inversely proportional to the square root of the particle concentration [P]:

$$d \propto 1/\sqrt{[P]} \quad (1)$$

Since particle concentration is inversely proportional to the volume, in the case of muscle the filament spacing within the living fibre is a function of the A-band volume (V_A) to which the thick filaments are confined by the smectic nature of the lattice:

$$d \propto \sqrt{V_A} \quad (2)$$

The explanation is straightforward. When interaxial separation is decreased by reduction of the available volume, the electrostatic repulsive forces which vary exponentially with separation⁹ are enhanced and must necessarily become greater than the Van der Waals' forces which vary inversely as the fifth power of distance⁹. Thus, the interaction energy of the system is

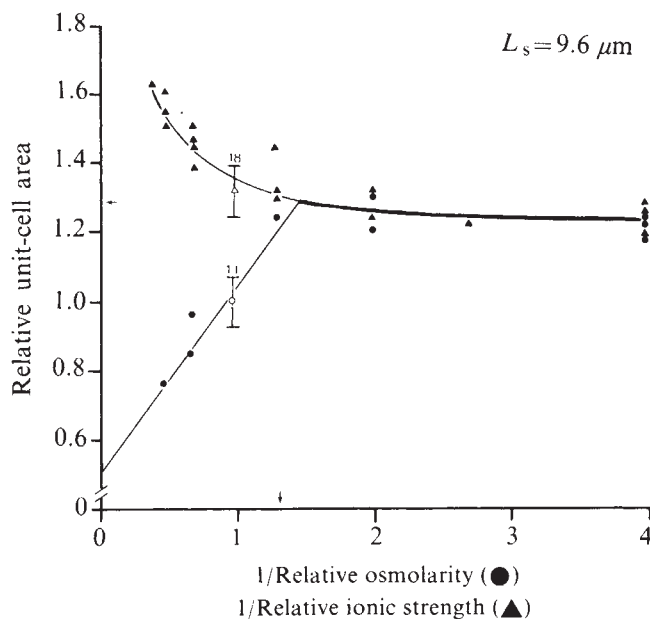


Fig. 3 Comparison of intact and skinned fibre lattice behaviour, recomputed and redrawn from April *et al.*¹⁵. The relative unit-cell areas of fibres in potassium propionate media are plotted against the reciprocal of the relative osmolarity for intact fibres (●) and the corresponding ionic strength for skinned fibres (▲). The lines are fitted by the method of least squares ($r_{\bullet} = 0.94$; $r_{\blacktriangle} = 0.90$). The open symbols represent mean values with the number of experiments and the standard deviations from the mean indicated. The divergent behaviour of the two lattices below a relative inverse osmolarity (or corresponding A-band volume) of approximately 130% of the control signifies different liquid-crystalline conditions. Above 130%, the behaviour of the intact fibre lattice is similar to that of the skinned fibre, indicating a change in the lattice of the intact fibre from a volume-constrained to an electrically balanced liquid-crystalline condition.

increased. If additional volume becomes available, the filaments move apart uniformly with the additional interaction energy (increased electrostatic forces) responsible for this readjustment. When interfilament distance increases to the point where opposing electrical forces balance, the interaction energy is minimal and further movement ceases unless pH or ionic strength of the medium is altered^{3,7,13,15}.

In other words, the lattice expands to fill the A-band until an equilibrium between Van der Waals' and electrostatic forces is reached.

The volume-limiting effect of the sarcolemma in the living fibre is equivalent to the performance of osmotic work whereby the interaction energy of the system is increased through confinement of the thick filaments to a volume less than that necessary for electrical force balance. It should be noted that in the volume-constrained condition the osmotic pressure counterbalances the electrostatic repulsive forces and relegates the Van der Waals' forces to a minor role in the maintenance of lattice stability. Thus, in the volume-constrained condition representative of living muscle, the fibre volume determines the maximum possible thick filament separation and, thus, the magnitude of the electrostatic forces. Within this confine the electrostatic forces between the thick filaments maintain the hexagonal lattice structure by mutual repulsion.

The volume-constrained liquid-crystalline condition of living single muscle fibres and the electrically balanced liquid-crystalline condition of skinned fibres can be compared in Fig. 3, in which the relative unit-cell areas of the lattices are plotted against the relative osmolarity and corresponding ionic strength of the bathing medium at pH 7.4. Both preparations were in identical media and the change in osmolarity or ionic strength was accomplished by adjusting the potassium propionate concentration of the relaxing solution. Clearly the behaviours of the filament lattices are different. The interaxial spacing in the skinned fibre is sensitive to the ionic strength of the medium whereas the interaxial spacing of the intact fibre is sensitive only to osmotically induced volume changes. In addition, on removal of the sarcolemma from a living fibre in conditions where the external ionic strength matches that of the sarcoplasm, the lattice immediately expands to the dimensions observed for the electrically balanced lattice at that particular ionic strength and pH. This indicates that the physiological volume of the living muscle fibre is inadequate for an equilibrium between van der Waals' and electrostatic forces and that such a condition is achieved after skinning the fibre.

Another inference from the data presented in Figs 2 and 3 is that as the intact fibre swells a point is reached where the filament lattice ceases to expand while the fibre as a whole continues to swell. This point represents a transition from a volume-constrained to an electrically balanced liquid-crystalline lattice because, once this point is reached, the lattice no longer exhibits a volume dependence and the behaviour becomes similar to that of the skinned fibre with respect to changes in ionic strength. Thus by sufficient swelling the volume constraint on the lattice can be removed. From this it follows that the spacing of the lattice in the intact fibre is determined by the condition that it fills the available volume until the spacing is sufficient for Van der Waals' attractions to balance electrostatic repulsion. Consequently, the previous calculations by Elliott *et al.*⁵, from which they concluded that lattice spacing was determined solely by the balance of Van der Waals' and electrostatic forces, cannot be applied to living muscle in physiological solution and their explanation for constant-volume shortening must be incorrect.

The principal conclusion to be drawn is that the thick filament lattice of living muscle is normally constrained and is sensitive only to changes in A-band volume. In contrast, the lattice state of glycerinated muscle, skinned fibres and greatly swollen fibres is electrically balanced *vis-à-vis* van der Waals' attractive and electrostatic repulsive forces and is sensitive to any parameter which affects electrostatic charges.

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ERNEST W. APRIL

Department of Anatomy,
College of Physicians and Surgeons
of Columbia University,
New York City, New York 10032

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- ¹ Needham, J., *Biochemistry and Morphogenesis*, 661-667 (Cambridge University Press, Cambridge, 1950).
- ² Elliott, G. F., and Rome, E. M., *Molec. Crystals liquid Crystals*, **8**, 215-219 (1969).
- ³ Rome, E., *J. molec. Biol.*, **37**, 331-344 (1967).
- ⁴ Elliott, G. F., *J. theor. Biol.*, **21**, 71-87 (1968).
- ⁵ Elliott, G. F., Rome, E., and Spencer, M., *Nature*, **226**, 417-420 (1970).
- ⁶ Miller, A., and Woodhead-Galloway, J., *Nature*, **229**, 470-473 (1971).
- ⁷ April, E. W., *J. Mechanochem. Cell Motility*, **3**, 000-000 (1975).
- ⁸ Moisesescu, D. G., *Bull. Mathematical Biol.*, **35**, 565-575 (1973).
- ⁹ Brenner, S. L., and McQuarrie, D. A., *Biophys. J.*, **13**, 301-331 (1973).
- ¹⁰ Brenner, S. L., and McQuarrie, D. A., *J. theor. Biol.*, **39**, 343-361 (1973).
- ¹¹ Mitchell, D. J., Ninham, B. W., and Richmond, P., *Biophys. J.*, **13**, 359-384 (1973).
- ¹² Parsegian, V. A. P., *A. Rev. Biophys. Bioengng.*, **2**, 221-256 (1973).
- ¹³ Matsubara, I., and Elliott, G. F., *J. molec. Biol.*, **72**, 657-669 (1972).
- ¹⁴ April, E. W., Brandt, P. W., and Elliott, G. F., *J. Cell Biol.*, **51**, 72-82 (1971).
- ¹⁵ April, E. W., Brandt, P. W., and Elliott, G. F., *J. Cell Biol.*, **53**, 53-65 (1972).
- ¹⁶ Bernal, J. D., and Fankuchen, I., *J. gen. Physiol.*, **25**, 111-165 (1941).

Low doses of DNP-D-GL, a potent hapten-specific tolerogen, are immunogenic *in vitro*

THE DNP conjugate of a D-amino acid copolymer of glutamic acid and lysine (DNP-D-GL) is a potent inducer of DNP-specific tolerance *in vivo* and *in vitro*^{1,2}. Simultaneous administration of DNP-D-GL and allogeneic cells to mice, however, results in anti-DNP antibody formation rather than tolerance³. DNP-D-GL thus seems to block activation of DNP-specific precursor cells in ordinary circumstances but to activate cells altered by allogeneic effect factors⁴. We have attempted to define further the conditions in which DNP-D-GL can induce antibody formation, a question made more relevant by the proposal that nucleic acids coupled to D-GL may be useful in preventing autoimmune disease, a situation where induction of antibody formation rather than tolerance would be unfortunate.

We have investigated the conditions in which DNP-D-GL can act as a tolerogen or an immunogen for T-independent anti-DNP responses of mouse spleen cells *in vitro*. Nanogram quantities of DNP-D-GL induced anti-DNP antibody formation, while larger amounts prevented responses to DNP-conjugates, as previously reported². Presentation of the DNP-D-GL in the form of antigen-pulsed macrophages further increased its immunogenicity and (or) decreased its tolerogenic potential. DNP-D-GL-induced anti-DNP antibody in nude mouse spleen

Table 1 *In vitro* anti-DNP PFC response of *nu/nu* and *nu/+* spleen cells to DNP₇₅-D-GL

DNP ₇₅ -D-GL ($\mu\text{g ml}^{-1}$)	Mean anti-DNP IgM PFC per culture $\pm$ s.e.*	
	<i>nu/nu</i> spleen cells	<i>nu/+</i> spleen cells
10^{-6}	25 $\pm$ 8	68 $\pm$ 29
10^{-5}	167 $\pm$ 23	87 $\pm$ 24
10^{-4}	189 $\pm$ 42	152 $\pm$ 38
10^{-3}	291 $\pm$ 99	272 $\pm$ 46
10^{-2}	262 $\pm$ 63	96 $\pm$ 54
10^{-1}	137 $\pm$ 30	0
10^0	0	0
10^{-2} DNP ₃₉ -AECM-Ficoll	1,023 $\pm$ 291	1,025 $\pm$ 98
SRBC	25 $\pm$ 5	890 $\pm$ 90

*Numbers are the mean PFC specific for DNP (background subtracted) in three replicate experiments $\pm$ s.e. Each experiment used spleen cells from one *nu/nu* and one *nu/+* animal.

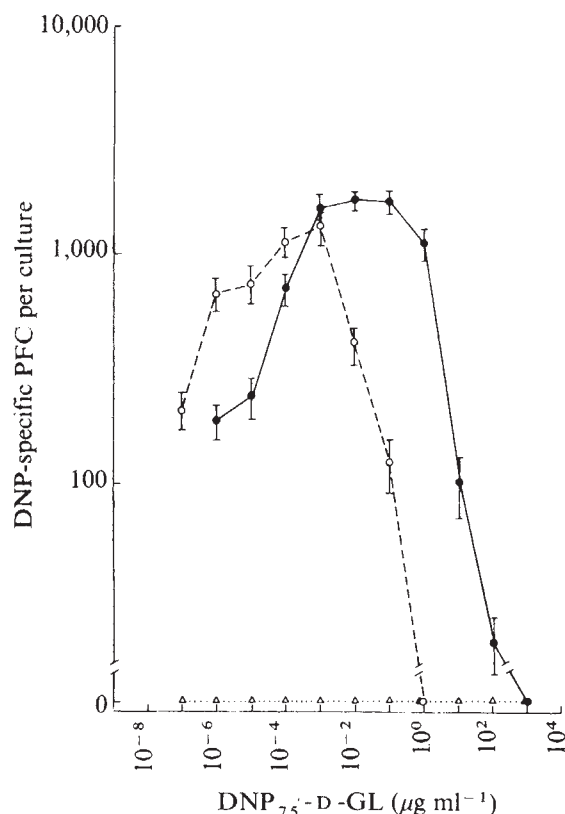


Fig. 1 The anti-DNP antibody response of BALB/c spleen cells cultured *in vitro* with DNP₇₅-D-GL. ○, Continuous DNP-D-GL added to normal spleen cells; ●, spleen cells exposed to indicated concentration of DNP-D-GL, washed, and antigen-pulsed adherent cells prepared. Normal non-adherent cells were added to the DNP-D-GL-pulsed adherent cells. △, Continuous DNP-D-GL added to macrophage-depleted non-adherent cells.

cell cultures. We conclude that DNP-D-GL is not intrinsically tolerogenic but can deliver both activating and inactivating signals to B lymphocytes, and that it is the concentration of DNP-D-GL and (or) the form of presentation which determines which of the two signals is dominant.

Spleen cells from BALB/c mice or from NIH outbred homozygous and heterozygous nude mice were cultured in Mishell-Dutton conditions as previously described⁵. DNP₇₅-D-GL (75 mol DNP per 105,000 daltons average molecular weight of D-GL, G:L ratio of 60:40) was added to cultures to give final concentrations from 10^{-8} to 10^4 $\mu\text{g ml}^{-1}$. DNP-D-GL-pulsed macrophages were prepared by incubating spleen cells with antigen for 1 h at 4 °C, washing three times, plating in culture dishes for 1 h at 37 °C, and washing the adherent cells twice more. Direct plaque-forming cells (PFC) were counted using TNP-coupled sheep erythrocytes⁶ as indicator cells. Induction of DNP-specific tolerance *in vitro* was assayed by the simultaneous addition of 10^{-3} $\mu\text{g ml}^{-1}$ DNP₃₉-aminoethylcarbonylmethyl (AECM)-Ficoll, a thymus-independent antigen *in vitro*⁵. Both DNP₇₅-D-GL and DNP₃₉-AECM-Ficoll were endotoxin-free by the *Limulus* amoebocyte lysate gelation assay⁷. Specificity of tolerance induction was shown by lack of depression of *in vitro* responses to sheep erythrocytes.

Figure 1 shows the mean anti-DNP response in three replicate experiments of spleen cells cultured with DNP₇₅-D-GL in three conditions: soluble antigen presented continuously to normal spleen cells; antigen-pulsed adherent cells (>90% macrophages) mixed in a 1:20 ratio with normal non-adherent (macrophage-depleted) spleen cells; or soluble antigen added directly to non-adherent cells. Soluble DNP-D-GL induced substantial anti-DNP-PFC on the part of whole spleen cell populations but failed to induce any detectable responses in non-adherent spleen cells. DNP-D-GL pulsed macrophages,

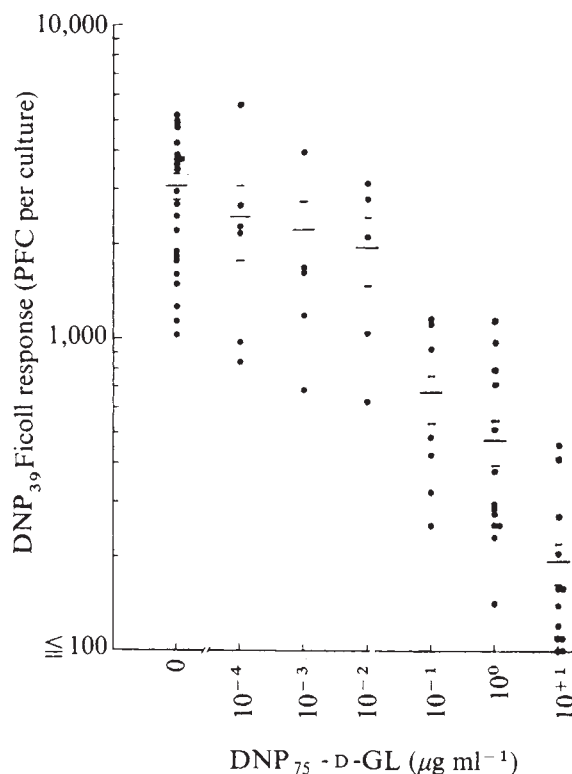
however, stimulated quite marked anti-DNP responses by non-adherent spleen cells. Since the amount of antigen retained by pulsed adherent cells was 1% or less, of the original dose indicated in Fig. 1 (as determined by retention of ³H-DNP-D-GL), the optimal amount of DNP₇₅-D-GL for inducing antibody production was 10^{-3} – 10^{-4} $\mu\text{g ml}^{-1}$ for both soluble antigen and pulsed macrophages.

The effect of immunogenic and suprainmunogenic concentrations of DNP-D-GL on the anti-DNP response of normal spleen cells to DNP₃₉-AECM-Ficoll is shown in Fig. 2. Data are pooled from 22 experiments with each point representing a group mean response in one experiment; the geometric mean $\pm$ s.e. is plotted. Immunogenic concentrations of DNP-D-GL (10^{-4} – 10^{-2} $\mu\text{g ml}^{-1}$) caused an insignificant reduction in the DNP-Ficoll response, but higher concentrations were highly suppressive of anti-DNP responses. This effect was antigen-specific in that parallel control responses to sheep red blood cells were not reduced by DNP-D-GL.

Spleen cells from individual outbred athymic nude mice responded to DNP-D-GL *in vitro* as well as spleen cells from phenotypically normal littermates (Table 1), although both had low anti-DNP PFC responses compared with BALB/c spleen cells (for example, Fig. 1). Nude spleen cells were susceptible to tolerance induction by DNP-D-GL; for example, $1 \mu\text{g ml}^{-1}$ reduced the DNP-Ficoll response from 1,204 to 240 PFC per culture. Induction of both antibody synthesis and DNP-specific tolerance thus seem to occur in mice lacking detectable T cell function.

These results indicate that DNP-D-GL can act as a T-independent antigen as well as a T-independent tolerogen in normal and nude mice. The response to DNP-D-GL does not occur when macrophage numbers are diminished, but it is not yet clear whether the remaining non-adherent lymphocytes are unable to respond to DNP-D-GL or are rendered tolerant by continuous exposure to soluble antigen. We conclude that the

Fig. 2 Inhibition of the anti-DNP response of BALB/c spleen cells to DNP₃₉-AECM-Ficoll by various concentrations of continuous DNP₇₅-D-GL. Each point is the mean response in a single experiment; data are pooled from 22 experiments. Horizontal bars indicate the geometric mean $\pm$ s.e.



poorly metabolisable nature of D-GL does not make it an obligate tolerogen, at least for normal mouse spleen cells in culture, and that its mechanism of tolerance induction may serve as a useful model for naturally occurring tolerance to antigens such as polysaccharides which display T-independent-like characteristics.

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DONALD E. MOSIER

Laboratory of Immunology,
National Institute of Allergy
and Infectious Diseases,
National Institutes of Health,
Bethesda, Maryland 20014

Received April 23; accepted July 24, 1975.

¹ Katz, D. H., Davie, J. M., Paul, W. E., and Benacerraf, B., *J. exp. Med.*, **134**, 201–233 (1971).

² Nossal, G. J. V., Pike, B. L., and Katz, D. H., *J. exp. Med.*, **138**, 312–317 (1973).

³ Osborne, D. P., Jr, and Katz, D. H., *J. exp. Med.*, **137**, 991–1006 (1973).

⁴ Armerding, D., and Katz, D. H., *J. exp. Med.*, **140**, 19–37 (1974).

⁵ Mosier, D. E., Johnson, B. M., Paul, W. E., and McMaster, P. R. B., *J. exp. Med.*, **139**, 1354–1360 (1974).

⁶ Rittenberg, M. B., and Pratt, C., *Proc. Soc. exp. Biol. Med.*, **132**, 575–581 (1969).

⁷ Elin, R., and Wolff, S., *J. infect. Dis.*, **128**, 349–352 (1973).

Continuous production of peroxidase, esterase, alkaline phosphatase and lysozyme by clones of promyelocytes

THE classic description of maturation of cells of the bone marrow myeloid series is based on static observation of morphological and biochemical differences between cells in close proximity¹. It is generally accepted that multipotential stem cells progress under appropriate leukopoietic stimuli to myeloblasts, and then to promyelocytes, myelocytes, metamyelocytes and at least three specific mature populations with distinct biological functions: polymorphonuclear neutrophils, basophils and eosinophils¹. The stage at which a cell becomes committed to a specific population is unknown. One approach to defining the maturation relationship between myeloid cells has been through study of the production of enzymes^{2–4}, chemical mediators⁵ and other intracellular or surface proteins^{6–9} specific to each leukocyte population. Histochemical techniques for identification of myeloid enzyme production have been used to correlate the production of these proteins with different stages of morphological maturation¹⁰. The inability to establish tissue culture lines derived from single myeloid cells stably arrested in development has prevented study of the degree of differentiation of cells in each

stage of maturation. We report here the establishment of multiple clones of rat myeloid cells which maintain stable promyelocyte morphology *in vitro* and produce four enzymes. Clones derived from more primitive myeloblast cells produce only lysozyme.

The Jones chloroma¹¹, a rat leukaemia line passaged more than 50 times *in vivo*, has maintained morphology typical of normal promyelocytes. Bone marrow samples obtained from affected rats are replaced with leukaemic cells which are positive in assays for esterase, alkaline phosphatase, peroxidase and lysozyme; however, fresh leukaemia cell explants or cells grown for short periods in tissue culture demonstrate enzyme positivity in only a fraction of cells tested by each histochemical assay (unpublished results). Similar observations are common when marrow is examined from a wide variety of experimental and human myelogenous leukaemias. This could reflect stable differences in enzyme production by different subpopulations within a leukaemia line or growth cycle-dependent variation in enzyme production by a truly homogenous population of cells. Another leukaemia line, the Wistar/Furth (W/Fu) acute myelogenous leukaemia (AML), has been shown to exhibit more primitive morphology and produce only lysozyme *in vivo* and *in vitro*¹². Here, 5–10% of cells consistently have seemed lysozyme-negative and could represent a subpopulation arrested in a developmental stage before that associated with lysozyme production.

To test these possibilities clonal lines were established in tissue culture from each transplantable tumour. These were maintained *in vitro* for around 2 months and then tested both for enzyme production and for morphological appearance. As Table 1 shows, each of 23 clones of the Jones chloroleukaemia produced peroxidase, esterase, alkaline phosphatase and lysozyme. Fifteen clones of W/Fu AML cells were indistinguishable in myeloblast morphology and production of a single enzyme, lysozyme. The proportion of positive cells in each assay did not differ significantly between clones tested.

Each clone of either line had a similar *in vitro* doubling time of 15–20 h, and saturation density of 3.0×10^6 – 4.0×10^6 cells ml⁻¹ in liquid medium. The clonal lines formed colonies with an efficiency of 5.0–5.5% in either liquid medium or semi-solid medium containing 0.9% methylcellulose. These *in vitro* growth parameters were indistinguishable from those of the parent lines.

The clonal lines were passaged continuously and tested periodically for changes in morphology and enzyme production and for evidence of C-type RNA virus production by assay for reverse transcriptase activity in tissue culture fluids¹³. The virus-specific nature of the enzyme was confirmed by inhibition

Table 1 Enzyme production by clonal lines of myeloid cells

Cell line	No. clones tested	No. clones positive (range of percent cells positive)			
		LAP	Esterase	Myeloperoxidase	Lysozyme
Jones promyelocytic leukaemia	23	23 (85–91)	23 (90–95)	23 (91–95)	23 (88–95)
W/Fu myeloblastic leukaemia	15	0	0	0	15 (91–96)

Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum (Colorado Serum Co.). The establishment of continuous tissue culture lines of the Jones chloroleukaemia and Wistar/Furth acute myelogenous leukaemia (W/Fu AML) have been reported¹². Multiple clonal lines derived from single cells of each leukaemia were selected by the microtitre plate technique according to published procedures¹⁹. Each was carried in tissue culture for 100 d. Histochemical assay methods for detection of myeloperoxidase, esterase and leukocyte alkaline phosphatase have been described¹⁰. A fluorescent antibody staining technique was used for detection of intracellular lysozyme. Cells were fixed and incubated with pre-absorbed rabbit serum, prepared against purified rat lysozyme, then washed and labelled with fluorescein-isothiocyanate conjugated, goat anti-rabbit globulin. Control preparations were incubated first with normal rabbit serum. Cells were scored using a $\times 1,000$ binocular microscope or fluorescent microscope by counting 1,000 cells in each of duplicate preparations for each clone.

with antibody to mouse C-type virus reverse transcriptase¹⁴. Several clones of the Jones chloroleukaemia were uniformly virus positive after 10 d in culture. The virus produced was shown to be C-type in origin by immunological tests for the interspecies antigenic determinant of the 30,000 molecular weight polypeptide of mammalian C-type virus¹⁵, and by electron microscopic analysis. It seemed likely that this was an endogenous rat virus. In spite of the continuous production of C-type virus for more than 300 d *in vitro*, there was no detectable change in promyelocyte morphology or in the strength of production of the four myeloid enzymes (data not shown). W/Fu AML cells remained virus-negative for several months in culture in spite of growth characteristics comparable with Jones chloroleukaemia cells. After 150 d *in vitro*, some W/Fu AML clones were found to be producing low levels of an indistinguishable rat C-type virus, yet there was no detectable change in myeloblast morphology or myeloid enzyme production.

Our results provide the first demonstration of the production of multiple lysosomal enzymes by clonal lines of myeloid cells and imply that all myeloid stem cells can produce multiple enzymes. The fact that only one myeloid enzyme was produced by W/Fu AML cells possessing a primitive myeloblast morphology, while several enzymes were synthesised by the more differentiated promyelocytic line, supports the finding in bone-marrow preparations of low or absent levels of myeloid enzymes in morphologically undifferentiated cells. If it is true that production of a single enzyme by a myeloblastic leukaemia results from maturation arrest rather than malignant transformation of a unipotent stem cell destined to produce only one enzyme, then stimulation of maturation of these myeloblasts should lead to production of other enzymes. These studies are in progress.

Both spontaneous¹⁶ and chemical^{17,18} activation of C-type viruses endogenous to murine cells in culture have been demonstrated previously. The present studies indicate that the spontaneous release of C-type virus by myelogenous leukaemias in culture occurred in the absence of any detectable morphological or biochemical alteration.

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JOEL S. GREENBERGER
STUART A. AARONSON

*Viral Carcinogenesis Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014*

DAVID S. ROSENTHAL
WILLIAM C. MOLONEY

*Department of Medicine,
Peter Bent Brigham Hospital,
Boston, Massachusetts 02115*

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- 1 Wintrobe, M. M., *Clin. Hematol.*, sixth ed. 224-282 (Lea and Febiger, Philadelphia, 1968).
- 2 Moloney, W. C., *Proc. eighth Cong. Eur. Soc. Haemat.*, 3, 14-15 (1962).
- 3 Moloney, W. C., Maniatis, A., *Proc. tenth Cong. Eur. Soc. Haemat.*, 2, 576-579 (1965).
- 4 Moloney, W. C., McPherson, K., and Fliegelman, L., *J. Histochem. Cytochem.*, 8, 200-207 (1960).
- 5 Gillespie, E., Lichtenstein, L. M., *J. clin. Invest.*, 51, 2941-2947 (1972).
- 6 Kulczycki, A., Jr, Iershy, C., and Metzger, H., *J. exp. Med.*, 139, 600-621 (1974).
- 7 Hawkins, D., *J. Immun.*, 110, 294-296 (1973).
- 8 Metzgar, R. S., Mohanakumar, T., and Miller, D. S., *Science*, 178, 986-989 (1972).
- 9 Halterman, R. H., Leventhal, B. G., and Mann, D. L., *New Engl. J. Med.*, 287, 1272-1274 (1972).
- 10 Moloney, W. C., *Cancer Res.*, 34, 3049-3057 (1974).
- 11 Jones, R. K., Brooks, A. L., Ferris, A. C., and Schaffer, D. K., *AEC Conf. 680529*, 517-532 (1970).
- 12 Greenberger, J. S., Rosenthal, D. S., Aaronson, S. A., and Moloney, W. C., *Blood*, 46, 27-38 (1975).
- 13 Scolnick, E. M., Rands, E., Aaronson, S. A., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, 67, 1789-1796 (1970).
- 14 Aaronson, S. A., Parks, W. P., Scolnick, E. M., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, 63, 920-924 (1971).

- 15 Stephenson, J. R., and Aaronson, S. A., *J. Virol.*, 12, 567-569 (1973).
- 16 Aaronson, S. A., Hartley, J. W., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, 65, 87-94 (1969).
- 17 Aaronson, S. A., Todaro, G. J., and Scolnick, E. M., *Science*, 174, 157-159 (1971).
- 18 Aaronson, S. A., and Dunn, C. Y., *Science*, 183, 422-424 (1974).
- 19 Stephenson, J. R., Reynolds, R. K., and Aaronson, S. A., *Virology*, 48, 749-756 (1972).

Spontaneous lymphokine synthesis by human blood mononuclear cells

LYMPHOCYTES, after antigenic stimulation, may synthesise and release biologically active soluble factors other than antibodies. These mediators were termed lymphokines by Dumonde¹, and the most extensively studied and best characterised are migration inhibitory factors which can inhibit the migration of macrophages or leukocytes: this is the property used for their *in vitro* bioassay. Apart from antigens, various other stimuli may trigger lymphokine synthesis by lymphocytes, for example, polyclonal mitogens², anti-immunoglobulin or membrane Fc or C3-receptor reactions^{3,4}. Furthermore, migration inhibitory activity has been found in the long term culture supernatants of some established lymphoid and even non-lymphoid cell lines^{5,6}. We report here that human blood mononuclear cells can, apparently without any inducing agent, produce material inhibiting migration of homologous leukocytes.

Mononuclear cells from healthy human individuals were obtained by Ficoll-Isopaque centrifugation of heparinised peripheral blood⁷, or by incubating sedimented leukocytes for 30 min at 37 °C with carbonyl iron and removing the phagocytic cells with a magnet. If not otherwise stated, 2×10^6 cells were cultured in 'universal' polystyrene containers (Sterilin, Richmond, Surrey) for 3 d in 1 ml of HEPES-buffered (10 mM) tissue culture medium 199 supplemented with 10% inactivated horse serum. Penicillin (70 IU ml⁻¹), streptomycin (70 µg ml⁻¹) and bicarbonate (0.1 M) were added to the medium, final pH 7.4. When puromycin was added to the control tubes its concentration was 5 µg ml⁻¹, which in previous tests was found to block the synthesis of migration inhibitory activity by cultured phytohaemagglutinin-stimulated cells. This concentration did not inhibit the effect of migration inhibitory activity on leukocytes and caused 10.7% inhibition of the migration of normal leukocytes in direct tests. The cell-free culture supernatants were tested for the presence of migration inhibitory activity with normal human blood leukocytes by the method of Clausen⁸. Each supernatant was tested in five or six replicate determinations. In our hands this method worked with standard deviations ranging from 2.2 to 11.8%, mean 6.6%.

In a series of experiments with mononuclear cells from 18 individuals, supernatants from unstimulated cultures produced 12.8% (mean) inhibition of migration when compared with migration areas of leukocytes in the corresponding medium. The phenomenon was not constant as significant inhibition (*t* test, paired samples) occurred in 11 out of 18 supernatants. In those 11 individuals the mean percentage of inhibition was 21.2 and the range was from 13.5 to 28.3%. That this was due to material actively synthesised by cultured cells is demonstrated in Table 1. The phenomenon did not occur if protein synthesis was blocked by cold or puromycin. The fact that, in the presence of puromycin, areas of migration were 9% smaller than in controls is explained by the direct effect of puromycin on leukocyte migration. Therefore, the use of puromycin controls in later experiments results in under- rather than overestimation of migration inhibitory activity.

Obviously, something in the conditions in which the cells remained during culture could stimulate the cells and bring about the synthesis of lymphokines. For example, foetal calf serum has been shown to be mitogenic for mouse B cells⁹. Therefore media supplemented with horse serum or autologous serum or no serum were compared for their possible stimulating activity (Fig. 1). The culture supernatants contained migration inhibitory activity regardless of the presence or nature of serum. Autologous serum did not affect inhibition. This indicates that

Table 1 Effect of blocking of protein synthesis on the generation of migration inhibitory activity in unstimulated culture supernatants

Cell donor	Medium control	37 °C	Supernatant of unstimulated cells cultured at 37 °C with puromycin	4 °C
1	25.2 ± 1.9*	18.8 ± 0.7	23.4 ± 2.7	
2	36.5 ± 3.1	29.1 ± 0.7	29.3 ± 0.9	
3	30.7 ± 2.6	23.0 ± 1.1	28.9 ± 2.5	
4	30.3 ± 1.4	23.9 ± 0.6	29.9 ± 2.2	
5	39.5 ± 1.4	28.3 ± 1.4	34.6 ± 1.5	
6	48.0 ± 5.7	40.6 ± 4.4	45.6 ± 4.4	
Mean	35.0	27.3	32.0	
		-----P < 0.01-----		
7	21.8 ± 0.8	18.1 ± 0.8		20.8 ± 0.4
8	23.9 ± 1.1	20.6 ± 0.5		27.0 ± 2.3
9	21.8 ± 0.8	15.2 ± 0.8		19.0 ± 1.0
10	23.9 ± 1.1	18.6 ± 1.3		25.8 ± 0.5
11	16.3 ± 1.1	13.9 ± 1.6		15.0 ± 1.2
Mean	21.5	17.3		21.5
		-----P < 0.02-----		

* Area of leukocyte migration in mm². Mean ± s.d. of five or six replicate determinations.

antibodies were not responsible for the occurrence of this phenomenon.

It is possible that the cells were stimulated during isolation and preparation before culture. Heparin was an unlikely stimulant because the phenomenon was also noticed in cultures of cells originating from blood samples defibrinated using glass beads. Another possibility was Ficoll-Isopaque. To investigate this question we separated mononuclear cells by Ficoll-Isopaque centrifugation, by the carbonyl iron method and by a combination of both. The results (Fig. 2) suggest that the stimulation takes place even in cultures not exposed to Ficoll-Isopaque.

The nature of the cells responding in those conditions was investigated by separating mononuclear cells by the sheep red

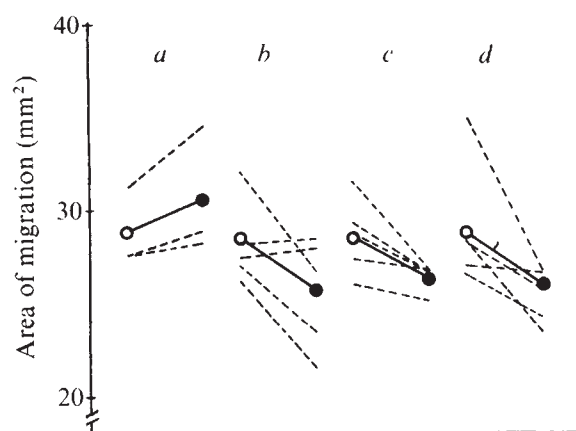
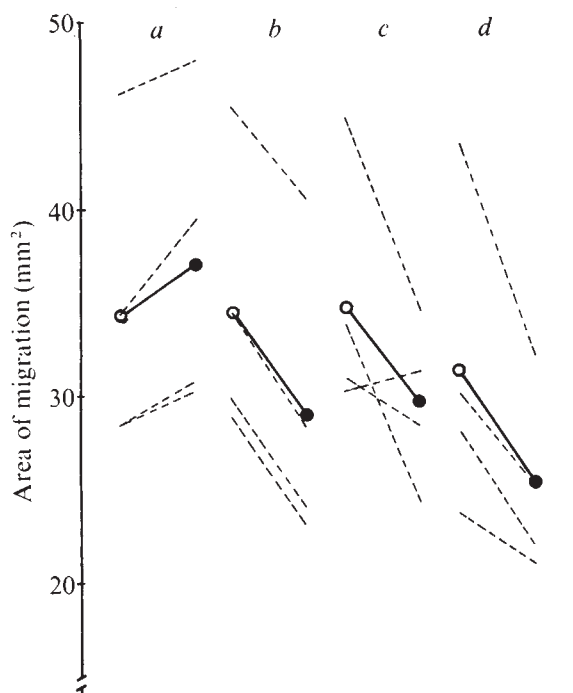


Fig. 2 Effect of the technique of isolation of mononuclear cells on the generation of migration inhibitory activity by unstimulated cells. For explanations, see legend to Fig. 1. *a*, Medium control; *b*, carbonyl iron; *c*, Ficoll-Isopaque | carbonyl iron; *d*, Ficoll-Isopaque.

Fig. 1 The role of serum as a possible cell stimulating agent. The cells were cultured with (○) or without (●) puromycin in the media indicated and the supernatants were tested with leukocytes for migration inhibitory activity. Medium control: leukocyte migration in the corresponding medium supplemented with horse serum, that is, not culture supernatant. -----, Individual experiments; ———, means. *a*, Medium control; *b*, horse serum; *c*, autologous serum; *d*, no serum.



blood cell-rosette technique¹⁰ into T cell-rich and B cell-rich populations. Preliminary experiments suggest that migration inhibitory activity is absent in T cell-rich cell supernatants and the responding cells are B cells and/or monocytes.

We have shown that this lymphokine-like activity in the supernatants of unstimulated cell cultures is brought about by an active synthetic process of cells and that its biological effect is indistinguishable from that of the migration inhibitory lymphokine. We have, however, no chromatographic data to support the conclusion that the phenomenon is caused by a definite migration inhibitory lymphokine. The synthesis of lymphokine(s) by apparently unstimulated human mononuclear cells in conventional culture conditions could be explained in several ways. First, the cells could have been stimulated already *in vivo* although, in our opinion, this is unlikely. Second, in theory, any of the steps in the isolation and preparation of cells could provide the necessary stimulus for the activation of cells. For example, guinea pig spleen B cells, after isolation on a nylon wool column can produce a chemotactic lymphokine without further stimulation⁴. Our experiments have failed so far to prove that any of the substances or procedures we used could act as a cell activating stimulus. Third, these cells may be activated during culture by the surface of the culture vessel on which they settle.

It has become popular to separate B and T lymphocytes and investigate their responses to mitogens, antigens or other

stimuli. In view of the various mechanisms of cooperation or amplification in the response of lymphocytes to these agents, our findings of mediator synthesis by a proportion of the mononuclear cells without evident stimulus should warrant careful interpretation of the claims of B or T cell specificity of various stimuli.

H. ARVILOMMI

Public Health Laboratory,
SF-40620 Jyväskylä 62, Finland

LIISA RÄSÄNEN

Department of Cell Biology,
University of Jyväskylä, Finland

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- ¹ Dumonde, D. C., *et al.*, *Nature*, **224**, 38 (1969).
- ² Pick, E., and Turk, J. L., *Clin. exp. Immun.*, **10** (1972).
- ³ Mackler, B. F., Altman, L. C., Rosenstreich, D. L., and Oppenheim, J. J., *Nature*, **249**, 834 (1974).
- ⁴ Wahl, S. M., Iverson, G. M., and Oppenheim, J. J., *J. exp. Med.*, **140**, 1631 (1974).
- ⁵ Granger, G. A., *et al.*, *J. Immun.*, **104**, 1476 (1970).
- ⁶ Papageorgiou, P. S., Henley, W. L., and Glade, P. R., *J. Immun.*, **108**, 494 (1972).
- ⁷ Boyum, A., *Scand. J. clin. Lab. Invest.*, **21**, Suppl. 97 (1968).
- ⁸ Clausen, J. E., *J. Immun.*, **110**, 546 (1973).
- ⁹ Andersson, J., and Melchers, F., *Progress in Immunology II*, **1**, 127 (1974).
- ¹⁰ Lay, W. H., Mendes, N. F., Bianco, C., and Nussenzweig, V., *Nature*, **230**, 531 (1971).

Anti-C3 antibodies in neonatal saliva

ANTIBODIES which react with bound complement components, in particular the third component C3, have been found in the parotid saliva of most normal subjects¹. These antibodies have several unusual properties². Their binding to complement is dependent on calcium ions, they can be inhibited by small amounts of native C3 and although they belong to the IgA class they are macromolecules with a sedimentation coefficient greater than 19S. The titres in mixed saliva are substantially lower than in parotid saliva¹ and the reduction in titre results from the presence of C3 in mixed saliva that combines with the antibody and neutralises its activity. While investigating the possibility that crevicular fluid was the source of C3 in the mouth, we examined samples of saliva from newborn babies. High antibody titres were found in these samples. We report here on the prevalence and properties of this antibody in neonatal saliva.

Whole saliva was collected from 36 babies, 21 male and 15 female, between 30 min and 4 h after birth and before the first milk feed. The saliva sample was collected by means of a small sterile sponge placed in the mouth between the gum and the cheek. The sponge was removed after a few minutes, weighed and mixed with a known volume of saline. After centrifugation the supernatant was separated and stored at -20°C . Antibody activity to complement was determined by a direct agglutination technique using sheep red blood cells coated with human serum¹. High agglutinating titres were found in all 36 samples, the mean titre being 1:3,000 (range 1:500–1:8,000). Agglutination of the cells was calcium dependent and could be reversed by addition of EDTA (final concentration 0.01 M).

The properties of this antibody were examined using saliva samples from six babies. Similar properties were found in all samples and these are summarised in Table 1. Fractionation of saliva on Sephadex G200 revealed that the activity was localised to the void volume fractions, indicating that the molecular weight of this antibody was greater than 10^6 .

Agglutination of complement-coated cells by antibody could be inhibited by small amounts of serum, by purified C3 and by a mixture of the C3 products, C3c and C3d. The inhibitory activity of the C3c and C3d mixture remained unaltered after heating at 56°C for 30 min (conditions which abolish the antigenicity of the C3d fragment)³. Some activity against the C3d fragment could be demonstrated by using the red cells obtained from a patient with cold agglutinin disease; these cells were agglutinated at low titre (1:2–1:8) by half of the neonatal saliva samples tested.

Table 1 Properties of anti-C3 antibodies in neonatal saliva

Inhibition by 0.01 M EDTA	+++
Inhibition by whole serum	+++
Reaction with zymozan	—
Sensitivity to trypsin	+
Inhibition by N-acetyl-D-glucosamine	—
Inhibition by mercaptoethanol	+++

+++ , Strong; + , weak; — , absent.

The concentration of IgA in saliva begins to rise a few days after birth^{4,5}. Very little information is available, however, on the concentration of immunoglobulin in saliva collected within a few hours of birth. The concentration and class distribution of immunoglobulin in neonatal saliva was, therefore, determined in 14 samples using a modified radial immunodiffusion assay. This was performed as described previously⁶ except that monospecific anti-immunoglobulin sera were incorporated into 0.8% 1-mm thick agar plates at a dilution of 1 in 80 and the samples were placed in 3-mm wells and allowed to react for 48 h at 37°C . The lowest detectable concentrations by this method were $2\text{ }\mu\text{g ml}^{-1}$ for IgG and IgM and $1\text{ }\mu\text{g ml}^{-1}$ for IgA. IgM and IgG were present in all unconcentrated samples of neonatal saliva. IgA, however, was detectable in only five of the 14 samples. The mean values and concentration range of the three immunoglobulins in saliva are summarised in Table 2. The distribution of the anti-C3 antibody within the immunoglobulin classes was determined using purified antibody. Pooled neonatal saliva (20 ml) was incubated with complement-coated red cells and after washing, the antibody was eluted from the cell surface by 0.01 M EDTA. The eluate was further concentrated tenfold by ultrafiltration and the concentration of immunoglobulin was determined by radial immunodiffusion. IgA was the only immunoglobulin detectable in the concentrated eluate.

The antibody titres found in neonatal saliva are three to five times higher than the titres found in the parotid saliva of normal adults. The molecular weight, immunoglobulin class and properties of both of these antibodies, however, seem to be identical. Both antibodies are also readily inhibited by native C3, a finding which contrasts markedly with the specificity shown by the serum antibodies which react with fixed complement. Serum immunoglobulins react with the hidden determinants in the complement molecule that are exposed on fixation and they are only weakly inhibited by native C3, whereas salivary antibodies react with the antigens present on native C3. It has been suggested that the anti-C3 antibodies in saliva arise following immunisation, possibly with bacteria which cross react with native C3 (ref. 7). The presence of very high titres in neonatal saliva within a few hours of birth, at a time when bacterial colonisation of the mouth is only beginning to take place⁸, suggests, however, that these antibodies are not produced by the foetus in response to immunisation with oral bacteria. The finding of this antibody in amniotic fluid at 34 weeks supports this view (unpublished results).

Table 2 Concentration of IgG, IgA and IgM in neonatal saliva

	IgG	IgM	IgA
Number positive	14/14	8/8	5/14
Mean ($\mu\text{g ml}^{-1}$) $\pm$ s.e.	14.2 ± 4.5	4.75 ± 1.2	$3.8 \pm 2.0^*$
Range	2–64	2–10	1–11

* Mean of five positive samples.

Attention has already been drawn to the possible relevance of these antibodies to the understanding of the nature of immunological tolerance⁷. Their occurrence in saliva implies that a population of B cells persists during foetal development and continues to survive into neonatal and adult life. The reasons for the persistence of the cells in salivary tissue are not

known but may be related to local factors or possibly to their requirement for calcium ions before binding to C3 occurs.

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J. F. PRICE*
B. D. WILLIAMS
S. J. CHALLACOMBE

Departments of Paediatrics, Medicine and Oral Immunology,
Guy's Hospital,
London SE1 9RT, UK

Received June 25; accepted July 24, 1975.

*Present address, Hospital For Sick Children, Great Ormond Street, London WC1, UK.

¹ Williams, B. D., Challacombe, S. J., Slaney, J. M., Lachmann, P. J., and Lehner, T., *Clin. exp. Immun.*, **19**, 423 (1975).

² Lachmann, P. J., and Thomson, R. A., *Immunology*, **18**, 157 (1970).

³ West, C. D., Davies, N. C., Fornistal, J., Herbst, J., and Spitzer, R., *J. Immun.*, **96**, 650 (1966).

⁴ Selner, J. C., Merrill, D. A., and Claman, H. N., *J. Paediatrics*, **72**, 685 (1968).

⁵ Tomasi, T. B., and Bienenstock, J., *Adv. Immun.*, **9**, 2 (1968).

⁶ Challacombe, S. J., *Caries Res.* (in the press).

⁷ Lachmann, P. J., Elias, D. E., and Moffett, A., in *Biological Activities of Complement* (edit. by Ingram, D. G.) (Karger, Basel, 1972).

⁸ McCarthy, C., Snyder, M. C., and Parker, R. B., *Archs oral Biol.*, **10**, 61 (1965).

Antisera to human B-lymphocyte membrane glycoproteins block stimulation in mixed lymphocyte culture

HUMAN B cells are frequently differentiated from T cells by the ready demonstration of immunoglobulin (Ig) on their surfaces¹. In the mixed lymphocyte reaction (MLR) the responding cells are mainly T cells²⁻⁴, whereas B cells have been suggested to be the primary stimulating cells⁵. The effect on the MLR of a specific anti-B-cell serum is therefore of considerable interest. Here we describe rabbit antisera to membrane glycoproteins of cultured human B cell lines which react only with Ig-bearing peripheral blood lymphocytes and which completely block the MLR *in vitro*.

During attempts to make xenoantisera to HL-A antigens, rabbits were immunised with papain-solubilised HL-A antigens purified from the human B cell line RPMI 4265 by previously reported techniques^{5,6}. Subsequently, the antisera were examined to determine the nature of the membrane molecules with which they reacted. The method of analysis involved incubating these antisera with detergent-solubilised membranes⁷ from cultured human lymphoblastoid lines (labelled with radioactive amino acids^{8,7}), precipitating the immune complexes with goat anti-rabbit IgG, and subjecting the washed precipitates to acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS)⁸. When tested against solubilised membranes derived from B cell lines (in this case, Ig-secreting lines), two of the sera reacted with molecules with apparent molecular weights of 35,000 and 27,000, in addition to what we presumed were HL-A glycoproteins (43,000) and β_2 -microglobulin (11,000)^{7,9-12}. When the sera was tested with labelled material from a human cell line (HSB) with T cell characteristics^{13,14}, however, only 43,000 and 11,000 molecular weight peaks were evident. Figure 1a shows profiles obtained on SDS-gel electrophoresis of a mixture of two immune precipitates. One was formed by incubating one of the sera (serum C) with ¹⁴C-amino acid-labelled, detergent-solubilised membranes from HSB, and the other by incubating serum C with ³H-amino acid-labelled membranes identically solubilised from the cell line SB. SB is an Ig-secreting cell line derived from the same donor as HSB^{13,14}. The additional molecules detected in the SB preparation can be seen clearly. The species with molecular weights 35,000 and 27,000 can also be precipitated from soluble membranes of B cell lines labelled with ³H-glucosamine, under conditions where β_2 -microglobulin (known not to contain carbohydrate¹⁵) is not labelled^{5,7}. This suggests that the molecules on B cell lines are glycoproteins.

In an attempt to make a specific reagent against these

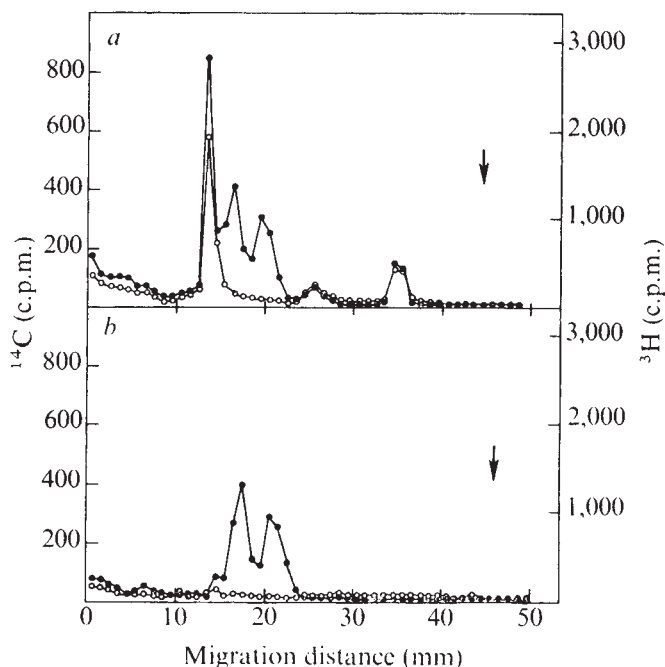


Fig. 1 SDS-gel electrophoresis profiles of immune precipitates of ¹⁴C-amino acid-labelled HSB membranes solubilised in 0.1% Nonidet P-40 (NP-40), 0.15 M NaCl, 0.01 M Tris, pH 8.0 (○), and ³H-amino acid-labelled SB membranes similarly solubilised (●). *a*, A mixture of precipitates formed with each preparation and serum C unabsorbed; *b*, a mixture of precipitates formed with each preparation and serum C absorbed three times (30 min, 25 °C) with 25×10^6 HSB cells per 100 μ l of serum. Insoluble material was removed from detergent-treated membranes³⁻⁷ by centrifugation at 100,000g for 90 min. An aliquot (25 μ l) of solubilised membranes (200,000 c.p.m.) was incubated with 1.5 μ l of serum for 1 h at 25 °C, and an excess of goat anti-rabbit IgG (adjusted to 0.1% NP-40) was added to effect precipitation. After 30 min at 37 °C, and a minimum of 1 h at 4 °C, the precipitates were washed three times with 0.1% NP-40 in 0.15 M NaCl, 0.01 M Tris, pH 8.0, twice with water, and subjected to electrophoresis on 13% SDS gels prepared according to Laemmli⁸. The gels were cut into 1-mm slices and counted as previously described^{5,7}. Calibration proteins used in molecular weight estimations were bovine serum albumin (67,000), ovalbumin (45,000), chymotrypsinogen A (25,000) and cytochrome *c* (12,000). The arrows indicate the position of the bromophenol blue marker dye.

glycoproteins, serum C was absorbed extensively with the T cell line, HSB. After absorption the serum failed to precipitate the 43,000 and 11,000 molecular weight peaks from the labelled membrane preparations (Fig. 1b). The reaction of the absorbed serum (serum C_{abs}) with the additional glycoprotein peaks in the SB preparation remained intact.

Serum C_{abs} was also examined for complement-mediated cytotoxicity of peripheral blood lymphocytes (PBL), using the ⁵¹Cr-release assay¹⁶. The results are shown in Fig. 2. Before absorption, serum C was highly lytic for human lymphocytes, giving 80% ⁵¹Cr-release, with a 50% release titre of 1 in 330. After absorption with HSB, approximately 25% ⁵¹Cr-release was observed (with a background release of 10%) to an antiserum dilution of 1 in 200 to 1 in 300. This experiment strongly suggests that the serum C_{abs} is cytotoxic for a minor population of peripheral lymphocytes.

The B cell specificity of the serum was demonstrated by two kinds of immunofluorescence study. In the first experiment, aliquots of PBL were incubated at 0 °C with serum C_{abs}, with a rabbit antiserum to human immunoglobulins (rabbit anti-human Ig, Nutritional Biochemicals), or with a mixture of the two sera. In all three cases both sera were diluted 1 in 5 in phosphate-buffered saline (PBS) containing 5% foetal calf serum with dinitrophenol added to 2.5 mM to prevent capping. Fluorescent cells were counted (at least 50 positive cells per sample) after washing and staining with a goat antiserum

to rabbit immunoglobulins conjugated with fluorescein isothiocyanate (FITC-goat anti-rabbit Ig, Meloy Laboratories). PBL treated with serum C_{abs} gave 18.5% positive fluorescence, those treated with rabbit anti-human Ig gave 16.6% positive fluorescence, and cells treated with a mixture gave 17.7% positive fluorescence. No summation was observed with the antiserum mixture, suggesting that both were reacting with the same population. Cells treated with a pre-immune bleed of serum C followed by FITC-goat anti-rabbit Ig showed only 1.3% positive fluorescence.

In a second experiment, designed to determine whether Ig-positive cells were lysed by serum C_{abs} , 10^7 PBL were incubated with 1 ml of the serum (diluted 1 in 30 in PBS, 30 min, 37 °C), centrifuged (1,000g, 5 min), resuspended in and incubated in 1 ml of rabbit complement (30 min, 37 °C). The cells were then applied to a discontinuous Ficoll-Hypaque gradient¹⁷ and centrifuged (2,000g, 30 min) to remove dead cells. The cells isolated from the interface (greater than 95% viable by Trypan blue staining) were washed and incubated with rabbit anti-human Ig or normal rabbit serum, followed by FITC-goat anti-rabbit Ig as described above. Lysed cells showed 1.7% positive fluorescence with normal rabbit serum and 2.5% positive fluorescence with rabbit anti-human Ig. Cells treated identically, but with normal rabbit serum and complement, showed 2.4% positive fluorescence with normal rabbit serum and 13.4% positive fluorescence with rabbit anti-human Ig. This experiment clearly shows that most (if not all) of the minor population of PBL lysed by serum C_{abs} are Ig-positive, that is, B cells.

Rabbit antisera to human β_2 -microglobulin (β_2m) have been reported to block the MLR in humans¹⁸, as have alloantisera to HL-A antigens^{19,20}. We have been conducting MLR blocking experiments with a rabbit antiserum to β_2m (ref. 7) and another to purified HL-A antigens solubilised by papain (serum A). These antisera react only with the 11,000 and 43,000 molecular weight HL-A-related species when assayed on B cell line labelled membranes by immune precipitation and SDS-gel electrophoresis as described in Fig. 2. These sera and serum C_{abs} were assayed for their ability to block the MLR. Figure 3 shows the results of these experiments. Clearly serum C_{abs} is the most effective at blocking the MLR. The response is reduced to background levels even at a dilution of 1 in 16,000 and is still approximately 50% inhibited at a dilution of 1 in 10^6 . Taking

Fig. 2 Lysis of peripheral blood lymphocytes by serum C (●), and serum C absorbed as in Fig. 1 with the T cell line HSB (○). Dilutions of the sera (10 μ l) were incubated (30 min, 37 °C) with 10 μ l of dextrose-gelatin veronal buffered saline (Grand Island Biological) containing 2×10^4 PBL (prepared by the method of Boyum¹⁴) labelled with $Na_2^{51}CrO_4$ (ref. 16). Rabbit complement (10 μ l) was added and the cells incubated for a further 30 min at 37 °C. After addition of 50 μ l of PBS containing EDTA (15 mM) and centrifugation, aliquots of the supernatants were counted. One hundred per cent release is the total c.p.m. incorporated into 2×10^4 cells, and the dashed line indicates the ^{51}Cr -release (10%) when no antiserum was added.

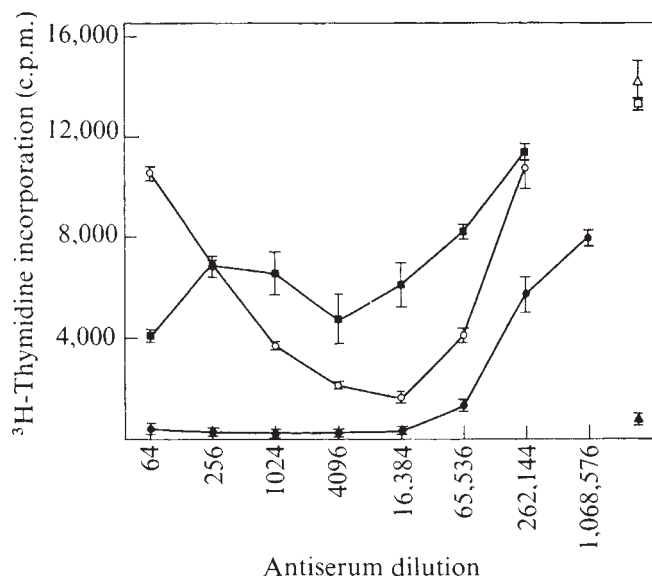
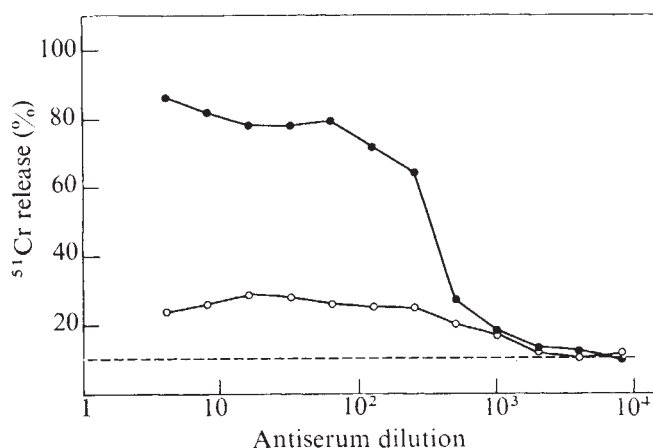


Fig. 3 Effect of various sera on the mixed lymphocyte response: serum C absorbed with HSB (●); serum A, a rabbit anti-HL-A (papain-solubilised) serum (○); rabbit anti- β_2 -microglobulin (■). Controls indicated are: responder cells plus stimulator cells in the absence of rabbit serum (□); responder cells alone (▲); responder cells plus stimulator cells with serum C pre-immune bleed at a dilution of 1 in 64 (△). Vertical bars indicate the standard error of the mean. Stimulator cells were treated with mitomycin C (40 μ g/ 10^7 PBL ml⁻¹) for 30 min at 37 °C. One way mixed lymphocyte cultures were set up in quadruplicate in U-well plates (Cooke Engineering). Responder cells (10^3 per well) and stimulator cells (10^3 per well) were incubated in 0.2 ml RPMI 1640, with 20% human type A serum and penicillin and kanamycin, containing the indicated dilutions of rabbit sera. All sera were heat-inactivated at 56 °C for 45 min. Cultures were collected using a Skatron cell harvester after 6 d at 37 °C in 5% CO₂ in air. 3H -Thymidine (1 μ Ci) was added per well 16 h before collection.

50% inhibition of the incorporation of 3H -thymidine as an endpoint, serum C_{abs} is one to two orders of magnitude more effective in blocking the MLR than rabbit anti-HL-A and anti- β_2m . The unusual shape of the curves for rabbit anti-HL-A and rabbit anti- β_2m seems to result from direct stimulation of the responder cells by the antisera. Controls with only responder cells and serum A or anti- β_2m added (not shown) gave increased 3H -thymidine incorporation at high concentrations of the sera. In similar controls no stimulation was observed by serum C_{abs} .

The fact that a serum which seems to be specific for B cells can block MLR is interesting, and may indicate that B cells are the predominant stimulator cells, as previously suggested⁴. In additional experiments (unpublished) we have shown that pretreatment of mitomycin C-treated stimulator cells with the anti-B-cell serum, followed by washing, blocks the MLR. Pretreatment of responder cells does not. We do not yet know, however, whether the serum reacts with other cells possibly involved in the MLR, such as macrophages, nor can we positively state that the antigens to which serum C_{abs} are directed are totally absent from T cells. They may be only operationally undetectable.

The nature of the papain-derived product which elicited the production of the B-cell-specific antibodies remains to be determined. At least four bands of apparent molecular weights between 20,000 and 32,000 appear on overloaded SDS gels of the antigen preparation used to produce serum C, in addition to the strong 34,000 and 11,000 molecular weight bands usually observed in papain-solubilised HL-A preparations^{5,6}. One or more of these species may be a papain cleavage product of the 35,000 or 27,000 molecular weight molecules. Experiments to characterise the immunogen(s) further are in progress. The possible relationship of the B cell glycoproteins to mouse Ia antigens, which are of similar molecular size²¹ and found

predominantly on B cells^{22,23} (though some are present on T cells²⁴) is also an area to be investigated. Other anti-human B-cell antisera have been reported^{25,26}, but the nature of the target antigens in these cases is unknown. A lytic antiserum specific for B cells with known target antigens promises to be a valuable reagent in human immunological studies.

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PETER CRESSWELL
STEVEN S. GEIER

Division of Immunology,
Duke University Medical Center,
Durham, North Carolina 27710

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- 1 Froland, S. S., and Natvig, J. B., in *Progress in Immunology*, 1 (edit. by Amos, D. B.), 107-110 (Academic, New York, 1971).
- 2 Johnston, J. M., and Wilson, D. B., *Cell. Immun.*, **1**, 430-444 (1970).
- 3 Mosier, D., and Cantor, H., *Eur. J. Immun.*, **1**, 459-461 (1971).
- 4 Plate, J. M. D., and McKenzie, I. F. C., *Nature new Biol.*, **245**, 247-249 (1973).
- 5 Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603-1607 (1973).
- 6 Turner, M. J., et al., *J. biol. Chem.*, **250**, 4512-4519 (1975).
- 7 Cresswell, P., and Dawson, J. R., *J. Immun.*, **114**, 523-525 (1975).
- 8 King, J., and Laemmli, U., *J. molec. Biol.*, **62**, 465-477 (1971).
- 9 Springer, T., Strominger, J. L., and Mann, D. L., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1539-1543 (1974).
- 10 Peterson, P. A., Rask, L., and Lindblom, J. B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 35-39 (1974).
- 11 Nakamuro, K., Tanigaki, N., and Pressman, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2863-2865 (1973).
- 12 Grey, H. M., et al., *J. exp. Med.*, **138**, 1608-1612 (1973).
- 13 Adams, R. A., Pothier, L., Hellerstein, E. E., and Boileau, G., *Cancer*, **31**, 1397-1407 (1973).
- 14 Kaplan, J., Mastrangelo, R., and Peterson, W. D., *Cancer Res.*, **34**, 521-525 (1974).
- 15 Bergaard, I., and Bearn, A. G., *J. biol. Chem.*, **243**, 4095-4103 (1968).
- 16 Sanderson, A. R., *Nature*, **204**, 250-253 (1964).
- 17 Boyum, A., *Scand. J. Clin. Lab. Invest.*, Suppl. 21 (97), 31-50 (1968).
- 18 Solheim, G., and Thorsby, E., *Tissue Antigens*, **4**, 83-94 (1974).
- 19 Ceppellini, R., et al., *Transplant. Proc.*, **3**, 58-70 (1971).
- 20 van Leeuwen, A., Schmit, H. R. E., and van Rood, J. J., *Transplant. Proc.*, **5**, 1539-1542 (1973).
- 21 Cullen, S. E., David, C. S., Shreffler, D. C., and Nathenson, S. G., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 648-652 (1974).
- 22 Sachs, D. H., and Cone, J. L., *J. exp. Med.*, **138**, 1289-1304 (1974).
- 23 Hämmerling, G. J., Deak, B. D., Maure, G., Hämmerling, V., and McDevitt, H. O., *Immunogenetics*, **1**, 68-81 (1974).
- 24 Frelinger, J. A., Neiderhuber, J., David, C. S., and Shreffler, D. C., *J. exp. Med.*, **140**, 1273-1284 (1974).
- 25 Greaves, M. F., and Brown, G., *Nature new Biol.*, **246**, 116-119 (1973).
- 26 Ishii, Y., et al., *J. Immun.*, **114**, 466-469 (1975).

Receptor-mediated inactivation of early B lymphocytes

THE interaction of surface immunoglobulin (sIg), the antigen receptor of B lymphocytes, with ligands—whether antigen or anti-Ig antibodies—initiates a series of surface and cytoplasmic events which, depending on the nature of the ligand and the presence or absence of cooperative signals from other cells, has various outcomes (reviewed in ref. 1). Immediately on the binding of a polyvalent ligand to sIg, the complexes redistribute on the cell surface, a contractile event occurs, the complexes are endocytosed and shed from the membrane and eventually new receptors appear on the cell surface¹. This *in vitro* cycle, in the absence of cooperative interactions, does not lead to B cell differentiation into secreting plasma cells, whereas it does so when induced in the proper conditions involving helper cells^{1,2}.

A crucial step in this ligand-induced cycle is the re-expression of new receptors on the cell surface, a process involving protein synthesis and requiring the activity of microtubules¹. It is expected that an interruption at this step, leading to a lack of receptors on the cell surface, would be tantamount to an inactive B cell unable to respond to antigen. We report here the failure of B cells from young mice to re-express sIg after relatively brief exposure to anti-Ig antibodies. This failure denotes a peculiar sensitivity of early B cells to ligand-receptor interaction in the absence of cooperative effects and could readily explain several of the phenomena of unresponsiveness

seen during the neonatal period. It should be noted that B cells from young mice (until about 2 weeks of age) have characteristics different from most adult B cells in that they lack C3 receptors^{3,4}, cap poorly⁴ and bind more labelled anti-Ig antibodies⁴.

The basic experiments consisted of exposing spleen lymphocytes from young (up to 2 weeks after birth) or adult (8-16 weeks) mice to rabbit anti-mouse Ig antibodies (RAMG) for 1 or 24 h, after which the cells were cultured for several days in the absence of RAMG. Surface Ig was detected by immunofluorescence. When RAMG bound to an adult B lymphocyte, sIg-anti-Ig complexes were eliminated within minutes, leaving the cell membrane cleared of sIg. Then, within a few hours of culture in the absence of anti-Ig, the cell restored its sIg, completing the process by 8-24 h later. The B lymphocyte from a young mouse spleen however, did not behave in this way. Depending on the time the cell was exposed to RAMG, the early B lymphocyte restored its sIg poorly or not at all. As Fig. 1 shows, within 1 d of a 1-h or a 24-h RAMG incubation (both of which completely cleared the cell of sIg), most of the original adult B lymphocytes restored their sIg. In contrast, only about half of the early B lymphocytes re-expressed sIg after a 1-h RAMG treatment, and almost none re-expressed it after 24 h of treatment with RAMG.

Since about half of the early B lymphocytes restored their sIg after a single cycle of clearance, it seems unlikely that the sIg initially on the cell surface represented cytophilic Ig. To strengthen this conclusion and to test whether early B lymphocytes can ever completely regenerate their sIg, cells of both ages were treated with the proteolytic enzyme Pronase at doses known to eliminate surface Ig, and then observed for the re-expression of sIg. Figure 2 shows that both early and adult B lymphocytes completely regenerated their sIg after with Pronase clearance. Note also for the early cells that a portion treated with Pronase after 24 h of incubation with RAMG did not re-express sIg. This shows that Pronase did not represent some stimulatory activity which might reverse the

Fig. 1 Re-expression of sIg after treatment with RAMG (the RAMG used was a poly-specific rabbit anti-mouse Ig antibody previously shown to bind only to B lymphocytes in the mouse spleen). Young (6-9 d, *a*) or adult (2-4 months, *b*) C57BL/6 mouse spleens were teased into Hanks' balanced salt solution (HBSS) plus 1% HEPES plus 5% foetal calf serum and washed three times. They were then exposed at a concentration of 10^7 cells per ml to Mishell-Dutton medium¹⁰ alone or containing RAMG (200 $\mu\text{g ml}^{-1}$) for 1 h or 24 h at 37°C in a 5% CO₂ atmosphere. The dose of RAMG was one that totally cleared the cells of sIg. After the RAMG treatments, cells were washed five times, resuspended at 10^7 cells per ml in fresh medium and cultured for various times. For immunofluorescence the cells were washed once, pelleted, resuspended in 50 μl of medium and 50 μl of fluoresceinated RAMG (FITC-RAMG) (500 $\mu\text{g ml}^{-1}$, F:P ratio approximately 6:3). The cells were incubated for 30 min at 4°C, washed twice, resuspended 10^7 per ml, and live cells were scored in a Leitz fluorescence microscope. —, Control; ---, 1-h RAMG; - - -, 24-h RAMG.

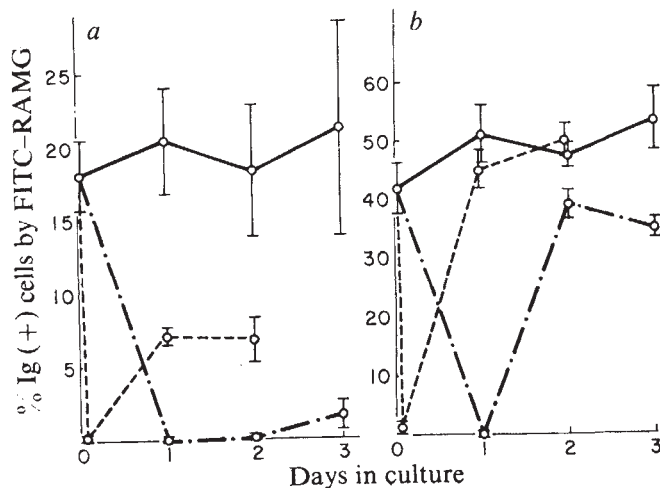


Table 1 Effect of RAMG treatment on LPS mitogenesis

Incubation (h)	Incubation medium	LPS in culture	Experiment 165 c.p.m. 10 ⁶ cells	E-C	E/C	Experiment 167 c.p.m. 10 ⁶ cells	E-C	E/C	Experiment 169 c.p.m. 10 ⁶ cells	E-C	E/C	Averages E-C	E/C
Young cells													
1	Medium	—	5,319 ± 97			7,605 ± 276			4,842 ± 407				
1	Medium	+	20,662 ± 1,047	15,343	3.88	46,455 ± 1,648	38,850	6.0	52,601 ± 808	47,759	10.86	33,964	6.95
1	RAMG	—	4,854 ± 138			6,770 ± 381			3,973 ± 338				
1	RAMG	+	5,787 ± 671	933	1.19	17,294 ± 271	10,524	2.55	17,406 ± 813	13,433	4.38	8,297	2.71
24	Medium	—	3,016 ± 362			5,339 ± 122			3,589 ± 189				
24	Medium	+	10,739 ± 278	7,724	3.56	15,085 ± 245	9,746	2.83	18,184 ± 696	14,595	5.07	10,688	3.82
24	RAMG	—	3,848 ± 157			7,393 ± 314			4,910 ± 189				
24	RAMG	+	2,083 ± 146	−1,765	0.54	4,456 ± 195	−2,937	.60	3,660 ± 225	−250	.94	−1,650	.69
Adult cells													
1	Medium	—	10,009 ± 654			10,979 ± 430			7,447 ± 220				
1	Medium	+	71,939 ± 2,033	61,930	7.19	77,002 ± 1,393	66,023	7.01	74,690 ± 1,180	67,243	10.03	66,065	8.08
1	RAMG	—	3,092 ± 288			3,792 ± 155			5,016 ± 56				
1	RAMG	+	35,960 ± 268	32,868	11.63	39,629 ± 561	35,847	10.45	62,899 ± 2,185	57,883	12.54	42,199	11.54
24	Medium	—	4,582 ± 117			6,972 ± 375			4,540 ± 300				
24	Medium	+	60,305 ± 412	55,723	13.16	59,311 ± 321	52,339	8.51	62,367 ± 1,027	57,827	13.74	55,296	11.80
24	RAMG	—	3,362 ± 692			2,180 ± 54			1,909 ± 68				
24	RAMG	+	61,059 ± 3,344	57,697	18.16	37,966 ± 1,660	35,786	17.42	55,392 ± 1,448	53,483	29.02	48,989	21.53

Young and adult C57BL/6 cells were incubated for 1 or 24 h in Mishell-Dutton medium containing RAMG (200 µg ml^{−1}) or no RAMG. They were then washed five times in HBSS plus HEPES plus FCS and resuspended to 10⁶ per ml in RPMI-1640 with penicillin and streptomycin and 5% FCS and 2 mM L-glutamine alone or with LPS (10 µg ml^{−1}). Each point was done in triplicate. After 24 h of culture in 5% CO₂, each tube was pulsed with 1 µCi in 50 µl of ³H-thymidine (2 Ci mmol^{−1}). The incorporation of the labelled thymidine into trichloroacetic acid-insoluble material was determined 24 h later. The results are expressed as the arithmetic mean of triplicate cultures ± s.e.m. E-C, Experimental mean—control mean; E/C, experimental mean/control mean.

effects of the 24-h treatment with RAMG. The main conclusion from the Pronase experiments is, therefore, that early B lymphocytes can re-express their sIg as completely and as rapidly as adult B lymphocytes but are specifically inactivated by interaction with RAMG.

The next question asked was whether these early B lymphocytes were inactivated or totally deleted from the cultures (that is, killed). To determine this, the spleen cells were examined after exposures to RAMG for the presence of another⁶,

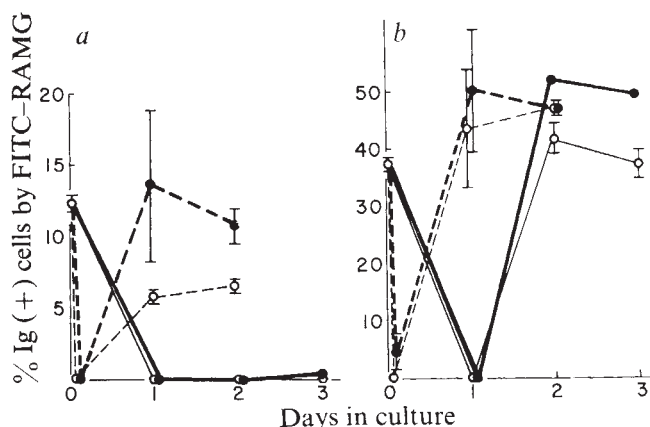
independent⁶ B lymphocyte marker, the I region-associated antigens (Ia). As all panels of Fig. 3 show, the surface distribution of Ia was unaffected immediately after the clearance of sIg by RAMG in early and adult B lymphocytes. One day after incubation with RAMG, adult B lymphocytes still showed their Ia and had restored their sIg. In contrast, early B lymphocytes also retained their Ia but had restored their sIg poorly or not at all. We concluded, therefore, that these early B cells were still present in the culture and alive at this time but had failed to re-express their sIg. Two days after the end of a 24-h treatment with RAMG the situation seemed to be changing, however. The percentage of early Ia-positive cells was declining, implying that, as a result of the prolonged ligand-receptor interaction, the original early B lymphocytes were either dying or were changing other cellular characteristics.

In addition to surface markers, the functional property of lipopolysaccharide (LPS)-induced mitogenesis was also studied after receptor clearance. Table 1 shows that the LPS mitogenic response of adult B lymphocytes was affected minimally, if at all, after treatment with RAMG. In striking contrast, early B lymphocytes had a marked reduction in LPS-induced mitogenesis after the same procedures. Thus, the failure to re-express receptors after RAMG clearance was accompanied by the loss of responsiveness to the potent B cell mitogen LPS.

Although the mechanism of this receptor-mediated inactivation of early B lymphocytes is not clear, certain conclusions can be drawn.

First, the inactivation seems to be a direct consequence of the interaction of RAMG with sIg and does not involve suppressor-type T-cell effects. In this system the RAMG only interacts with B lymphocytes. Furthermore, in experiments involving mixtures of adult and young spleen cells, each population reacts independently, neither one influencing the other in any direction. Second, this inactivation was not associated with proliferation of B cells after treatment with RAMG. We have failed repeatedly to detect any mitogenic

Fig. 2 Re-expression of surface Ig after treatment with Pronase. Young (6–8 d, a) and adult (2–4 months, b) C57BL/6 cells were treated with Pronase (2 mg ml^{−1}) (*Streptococcus griseus* protease) and DNase II (20 µg ml^{−1}) for 1 h at 37 °C in HBSS plus HEPES, washed five times and then cultured and assayed as in Fig. 1. Treatment with Pronase was either immediately after cell collection or after 24-h culture in Mishell-Dutton medium containing RAMG (200 µg ml^{−1}) and three washes. For comparison, re-expression after incubations of 1 and 24 h in Mishell-Dutton medium containing RAMG (200 µg per ml) are also shown. ○—○, 1 h RAMG; ○—○, 24 h RAMG; ●—●, 1 h Pronase; ●—●, 24 h RAMG, 1 h Pronase.



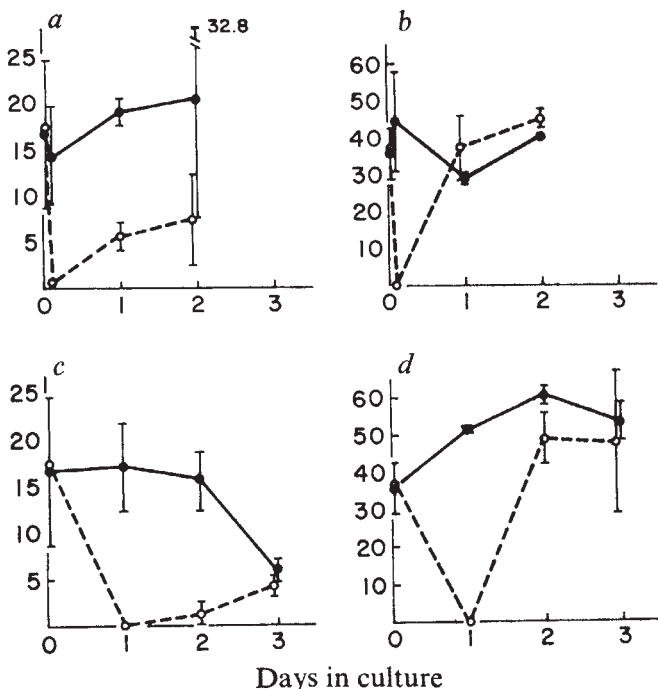


Fig. 3 Re-expression of surface Ig in relation to surface Ia. The four panels show young and adult AKR cells treated for 1 and 24 h with RAMG as in Fig. 1. For each assay point, a tube of cultured cells was washed once, pelleted, resuspended in 50 μ l rhodamine-conjugated RAMG (R-RAMG), (1.3 mg ml⁻¹), incubated for 30 min at 4°C, washed three times, and resuspended in 20 μ l normal mouse serum. Twenty microlitres fluorescein-conjugated ATH anti-ATL spleen cell serum (F anti-Ia) were added, and the cells were incubated for 30 min at 4°C, washed twice and resuspended to 10⁷ per ml. The cells were then scored for the presence of Ig (rhodamine stain) and Ia (fluorescein stain). *a*, Young cells, 1 h RAMG; *b*, adult cells, 1 h RAMG; *c*, young cells, 24 h RAMG; *d*, adult cells, 24 h RAMG. ●, % cells Ia (+) by FITC-Ia; ○, % cells Ig (+) by R-RAMG.

effect of RAMG on early or adult murine B lymphocytes. Finally, this receptor-mediated inactivation seems to be controlled by some intracellular mechanism independent of the presence or absence of ligand-receptor complexes on the membrane. Ault *et al.*⁷ showed that after binding a highly tolerogenic polypeptide of *d*-amino acids, primed adult mouse spleen cells did not regain antigen-binding capability. Also, the complexes were not totally cleared from the cell surface. One could explain these results⁷ by postulating a relationship between clearance of complexes and expression of new receptors. This explanation does not apply to our experiments, however, since the early B cell does clear its surface of complexes. Also, even if some long lasting complexes were present, the Pronase should have removed them and released the cell.

These results show that the interaction of the antigen receptors of an early B lymphocyte with a specific ligand leads to the inactivation of two fundamental B cell processes—receptor re-expression and LPS-induced mitogenesis. This phenomenon may be the basis for several situations of immunological unresponsiveness. The inactivation reported here could explain, in part, the phenomenon of allotype suppression, as well as the analogous cases of suppression of specifically committed cells by anti-receptor antibody^{8,9} and of cells bearing a particular Ig class by class-specific antibody¹⁰. Further, young animals are notoriously easy to render specifically tolerant towards antigen. Although other cells and mechanisms are probably also operative¹¹, a specific antigen may directly turn off the early B lymphocytes by the process discussed here. Studies are in progress evaluating this point directly on antigen-binding lymphocytes. Finally, the inactivation of early type B lymphocytes could also have a role in adult tolerance. There is

evidence that adult mouse spleen⁴ and bone marrow¹² contain some B lymphocytes of the type constituting the young spleen. These may be precursors which constantly repopulate the pool of competent adult B lymphocytes¹³. It is possible that the state of unresponsiveness in the adult, although complex, could be prolonged by the ligand-induced inactivation described here, having as its target the early type of B cell found in bone marrow. In this respect, it has been shown that adult bone marrow lymphocytes are very easily rendered tolerant by antigen *in vitro*¹⁴.

CHARLES L. SIDMAN
EMIL R. UNANUE

Department of Pathology,
Harvard Medical School,
Boston, Massachusetts 02115

Received June 16; accepted July 29, 1975.

- 1 Unanue, E. R., *Am. J. Path.*, **77**, 1–20 (1974).
- 2 Kishimoto, T., and Ishizaka, K., *J. Immun.*, **114**, 585–591 (1975).
- 3 Gelfand, M. C., Effenbein, G. J., and Paul, W. E., *J. exp. Med.*, **139**, 1125–1141 (1974).
- 4 Sidman, C. L., and Unanue, E. R., *J. Immun.*, **114**, 1730–1735 (1975).
- 5 Shreffler, D. C., and David, C. S., *Adv. Immun.*, **20**, 125–195 (1975).
- 6 Unanue, E. R., Dorf, M. E., David, C. S., and Benacerraf, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 5014–5016 (1974).
- 7 Ault, K. A., Unanue, E. R., Katz, D. H., and Benacerraf, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3111–3114 (1974).
- 8 Strayer, D. S., Cosenza, H., Lee, W. M. F., Rowley, D. A., and Köhler, H., *Science*, **186**, 640–643 (1974).
- 9 Köhler, H., Kaplan, D. R., and Strayer, D. S., *Science*, **186**, 643–644 (1974).
- 10 Lawton, A. R., Asofsky, R., Hylton, M. B., and Cooper, M. D., *J. exp. Med.*, **135**, 277–297 (1972).
- 11 Mosier, D. E., and Johnson, B. M., *J. exp. Med.*, **141**, 216–226 (1975).
- 12 Ryser, J. E., and Vassalli, P., *J. Immun.*, **113**, 719–728 (1974).
- 13 Osmond, D. G., *J. reticuloendothel. Soc.*, **17**, 99–114 (1975).
- 14 Nossal, G. J. V., and Pike, B., *J. exp. Med.*, **141**, 904–917 (1975).
- 15 Mishell, R. I., and Dutton, R. W., *J. exp. Med.*, **126**, 423–442 (1967).

In vitro* recognition of carcinogen-induced local denaturation sites in native DNA by S₁ endonuclease from *Aspergillus oryzae

It is known^{1–4} that the AAF-addition products obtained in the reaction of the ultimate carcinogen, N-acetoxy-N-2-acetylaminofluorene (AAAF) with DNA *in vitro* are essentially the same as those isolated from liver DNA of rats fed with N-2-acetylaminofluorene (AAF). Thus, the modifications of DNA secondary structure observed *in vitro* should reflect DNA alterations arising *in vivo*. It has been shown^{5–9} that the main alteration induced by the fluorene ring is the creation of locally disorganised regions inside the double-helical structure. We now show that S₁ endonuclease (EC3.1.4.) from *Aspergillus oryzae* is able to excise the local regions of denaturation induced in native DNA molecules by AAAF.

When the kinetics of the hydrolysis of a carcinogen-modified native DNA sample (DNA-AAF) by endonuclease were measured they were found to be intermediate between the kinetic curves of native and fully denatured DNA (Fig. 1*a*). Moreover, by following A₃₀₅, the acid-soluble material was shown to contain fluorene (Fig. 1*b*). Analogous measurements using native DNA modified by two derivatives of AAF are also reported.

The first, N-acetoxy-N-2-acetylaminofluorene (AAAF), is a model of the ultimate metabolite of the strong carcinogen, N-2-acetylaminofluorene (AAF)¹⁰. According to the general mechanism of activation of arylamides¹, the second, N-acetoxy-N-2-acetylaminofluorene (AAAF), may be a metabolite of the non-carcinogen N-2-acetylaminofluorene (AAIF)¹¹. A native DNA sample modified by the fluoro-derivative (DNA-AAAF) showed almost the same behaviour towards S₁ as DNA-AAF (Fig. 1*a*). On the other hand, hydrolytic attack on a DNA sample modified to the same extent with the iodo-derivative (DNA-AAIF) was much slower (Fig. 1). These results fit very well with those obtained previously using purely physicochemical experiments^{5–8}.

How the covalently linked fluorene residues induce local opening of the double helix may be viewed schematically as

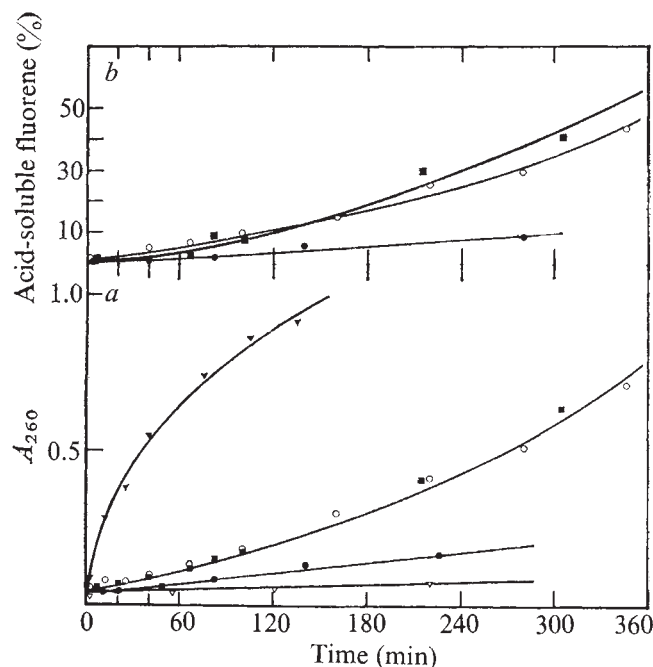


Fig. 1 Kinetics of hydrolysis of different calf thymus DNA samples by S_1 endonuclease from *Aspergillus oryzae*. ∇ , Native DNA; $\blacktriangledown$, heat-denatured DNA; $\circ$, DNA-AAF; $\blacksquare$, DNA-AAFF; $\bullet$, DNA-AAIF. DNA was prepared from calf thymus²³ and had the following characteristics: hypochromicity at 260 nm: 40%; $S_{20,w}$: 21S; ϵ_p^{260} : 6,420; protein content $\leq 0.8\%$ by weight. Denatured DNA was obtained by heating a DNA sample in a sealed vial to 100 °C for 10 min. To avoid DNA renaturation the sample was rapidly chilled in ice. Reaction of native DNA samples with the fluorene derivatives was carried out as described previously⁵. All fluorene-reacted DNA samples have approximately 5% of their bases modified. Hydrolysis experiments were carried out as described by Vogt in the following buffer: 0.03 M sodium acetate, pH 4.6, 0.05 M NaCl 1 mM $ZnSO_4$, 5% glycerol. DNA concentration was about 180 $\mu g\ ml^{-1}$. Enzyme was prepared according to Vogt²². According to the definition of one unit of nuclease activity²² our enzyme solution titrates at 18 U/10 μl . *a*, Concentrations of acid-soluble material were measured as follows: incubations, carried out at 45.0 ± 0.1 °C, were terminated by chilling and addition of 0.6 ml ice-cold 10% perchloric acid to 0.3 ml aliquots. Samples were centrifuged for 15 min at 5,000 r.p.m. and A_{260} determined. *b*, Percentage of acid-soluble fluorene was determined by recording A_{305} .

follows. The ultimate carcinogen (AAAF) reacts mainly at position C-8 on guanine residues^{12,13}. If polynucleotides are strictly held in a static double-helical structure, there is no possibility of chemical reaction at position C-8 of purines (and C-6 of pyrimidines). Thus, to enable a successful carcinogen attack, we must presume a dynamic state for native DNA¹⁴, with transiently open base pairs ('breathing units'), as proposed by von Hippel *et al.*¹⁵. A consequent conformational change brings the modified base from its *anti* conformation to a *syn* conformation by rotation around the glycosyl linkage¹⁶. The three-dimensional conformation, however, is not yet clear.

To explain the local denaturation sites induced by the fluorene ring indicated by the decrease in melting temperature^{5,14,17} and by the kinetics of formaldehyde unwinding^{6,7}, we have proposed an 'insertion-denaturation model' in which the fluorene ring is accommodated between the two nearest base plates⁵⁻⁸. This model has been confirmed by Weinstein *et al.*^{9,18}. Chan *et al.*¹⁹ measured the orientation of the fluorene in renatured DNA of T4 phage and found a value consistent with the AAF residue being constrained to lie along the helix-winding angle. I have, however, obtained measurements of electric dichroism showing the fluorene ring perpendicular to the helix axis in the case of DNA-AAF and DNA-AAFF (R.P.P.F, unpublished). On the other hand, for the iodo-derivative we found the angle

between the transition moment of fluorene at 305 nm and the helix axis to be 60°.

We may distinguish therefore two different addition products: denaturing addition products (DNA-AAF and DNA-AAFF), in which the fluorene ring gives rise to single-stranded-like regions; these regions are S_1 -sensitive; non-denaturing addition products (DNA-AAIF), in which the insertion of the fluorene ring is probably hindered (in this case by the bulky iodine atom). As local helix opening does not occur, little or no hydrolysis by the S_1 nuclease is detected. Work on the chemical nature of the addition products in DNA-AAFF and DNA-AAIF is in progress.

I stress the increased importance in chemical carcinogenesis of the reaction of cells to limit damage to DNA. Cellular repair mechanisms are a consequence of the local distortion or breakage of DNA strands²¹. Enzymes able to recognise and to excise distorted regions of DNA are probably involved in the earliest step of cellular repair.

At present there is no evidence for a correlation between the presence of locally disorganised groups of nucleotides in native DNA and the carcinogenic process. There is, however, a striking correlation between carcinogenic power of aromatic amides and the 'molecular thickness' of their aromatic ring²⁰. In most cases, if the aromatic ring is either non-planar or substituted by a bulky atom or group, the corresponding amide shows little or no carcinogenic activity.

Although AAIF was essentially inactive as a carcinogen, it does not necessarily follow that AAAIF is also non-carcinogenic. The parent compound may not be 'activated' (that is, N-hydroxylated and esterified) *in vivo*. Direct tests on the carcinogenicity of AAAIF are now in progress.

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ROBERT P. P. FUCHS

*Institut de Biologie Moléculaire et Cellulaire,
15 rue Descartes,
67000 Strasbourg, France*

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- 1 Miller, J. A., *Cancer Res.*, **30**, 559-576 (1970).
- 2 Kriek, E., *Chem. Biol. Interactions*, **1**, 3-7 (1969-70).
- 3 Kriek, E., *Cancer Res.*, **32**, 2042-2048 (1972).
- 4 Irving, C. C., and Veazey, R. A., *Cancer Res.*, **29**, 1799-1804 (1969).
- 5 Fuchs, R., and Daune, M., *Biochemistry*, **11**, 2659-2666 (1972).
- 6 Fuchs, R., and Daune, M., *FEBS Lett.*, **34**, 295-298 (1973).
- 7 Fuchs, R. P. P., and Daune, M. P., *Biochemistry*, **13**, 4435-4440 (1974).
- 8 Fuchs, R., and Daune, M., *FEBS Lett.*, **14**, 206-208 (1971).
- 9 Levine, A. F., Fink, L. M., Weinstein, I. B., and Grunberger, D., *Cancer Res.*, **34**, 319-327 (1974).
- 10 Miller, J. A., Sandin, R. B., Miller, E. C., and Rusch, H. P., *Cancer Res.*, **15**, 188-199 (1955).
- 11 Morris, H. P., Velat, L. A., Wagner, B. P., Dahlgard, N., and Roy, F. E., *J. natn. Cancer Inst.*, **24**, 149-180 (1960).
- 12 Kriek, E., Miller, J. A., Juhl, U., and Miller, E. C., *Biochemistry*, **6**, 177-182 (1967).
- 13 Miller, E. C., Juhl, U., and Miller, J. A., *Science*, **153**, 1125-1127 (1966).
- 14 Kapuler, A. M., and Michelson, A. M., *Biochim. biophys. Acta*, **232**, 436-450 (1971).
- 15 McConnel, B., and Von Hippel, P. H., *J. molec. Biol.*, **50**, 297-316 (1970).
- 16 Nelson, J. H., Grunberger, D., Cantor, C. R., and Weinstein, I. B., *J. molec. Biol.*, **62**, 331-346 (1971).
- 17 Troll, W., Rinde, E., and Day, P., *Biochim. biophys. Acta*, **174**, 211-219 (1969).
- 18 Weinstein, I. B., and Grunberger, D., in *World Symposium on Model Studies in Chemical Carcinogenesis* (edit. by Ts'o, P. O. P. and Di Paolo, J.), 217-235 (Dekker, New York, 1974).
- 19 Chang, C. T., Miller, J. J., and Wetmur, J. G., *Biochemistry*, **13**, 2142-2148 (1974).
- 20 Weisburger, E. K., and Weisburger, J. H., *Adv. Cancer Res.*, **5**, 331-431 (1958).
- 21 Cerutti, P. A., *Life Sci.*, **15**, 1567-1575 (1975).
- 22 Vogt, V. M., *Eur. J. Biochem.*, **33**, 192-200 (1973).
- 23 Kay, E. R. M., Simmons, N. S., and Dounce, A. L., *J. Am. chem. Soc.*, **74**, 1724-1726 (1952).

Localisation of satellite DNA sequences in nuclei and chromosomes of two plants

MANY eukaryotic DNAs can be resolved into two or more components by centrifugation to equilibrium in neutral $CsCl^{1,2}$, the minor components being called satellite DNAs. In addition to $CsCl$ satellites, minor portions of the genome have been

resolved from the main DNA band by differential binding with Ag^+ or Hg^{2+} ions, followed by centrifugation in Cs_2SO_4 .

The satellite DNA in mouse, isolated as either a CsCl satellite or as a Ag^+ complex, has been localised, by *in situ* hybridisation, over the chromocentres in interphase nuclei, and at the centromeres of all the metaphase chromosomes^{3,4}. Further satellites have been investigated in various animal species including *Drosophila*^{5,6}, *Rhynchosciara*⁷, the higher apes and man⁸. The small size of the chromosomes in plants containing CsCl satellite DNA⁹ has frustrated attempts to localise these sequences cytologically. We describe here the properties of a $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA from *Scilla sibirica*, a plant with large, morphologically distinguishable chromosomes containing heterochromatin¹⁰, and demonstrate by *in situ* hybridisation the nuclear and chromosomal location of these sequences. Some preliminary observations are presented for an $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA isolated from *Vicia faba*.

The nuclear DNA of *S. sibirica* forms a slightly asymmetrical band with a buoyant density of 1.701 g cm^{-3} on centrifugation in CsCl (Fig. 1a). When the DNA is complexed with Ag^+ ions a light satellite, comprising 5–10% of the total DNA is completely resolved from the bulk of the DNA on centrifugation in Cs_2SO_4 (Fig. 1b). After purification of this band on preparative $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ gradients the satellite DNA forms a single homogeneous band at 1.705 g cm^{-3} in CsCl (Fig. 1c). This $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA probably accounts for the asymmetry of the total DNA band in Fig. 1a.

Figure 2a shows the thermal denaturation curve of *S. sibirica* satellite DNA in $0.25 \times \text{SSC}$ (0.04 M NaCl and $0.004 \text{ M Na-citrate}$, $\text{pH } 7.2$). The profile reveals at least three components of very different C+G composition, separable by thermal denaturation but apparently similar in buoyant density in CsCl . The major melting component (60%), with a T_m of 91.5°C in $0.25 \times \text{SSC}$, corresponds to a high C+G composition of about 75%. Figure 2b shows that the renaturation kinetics of the satellite in $0.25 \times \text{SSC}$ are at least biphasic. Estimation of the second order rate constant for the fast component, assuming that this contributes 60% of the total satellite DNA, gives a value of $780 \text{ mol}^{-1} \text{ s}^{-1}$ in $0.5 \times \text{SSC}$. This is very similar in

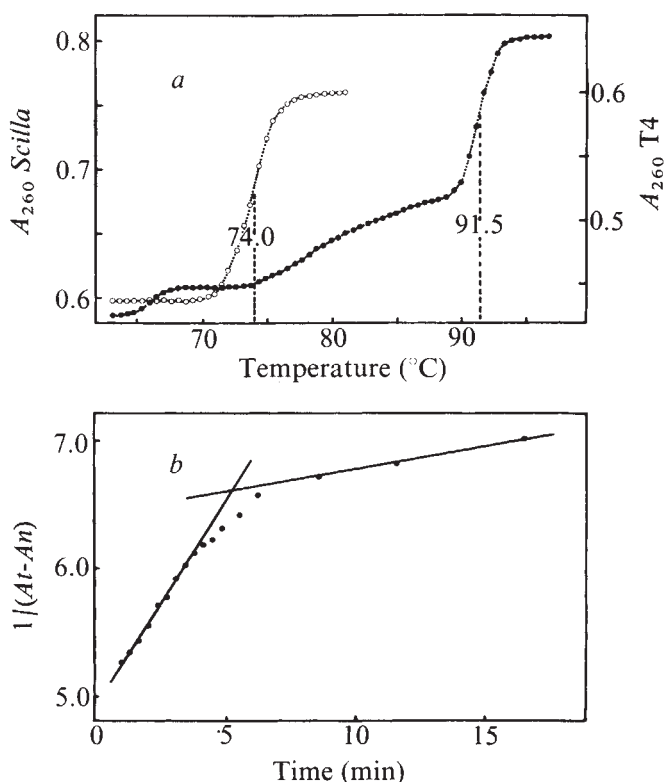
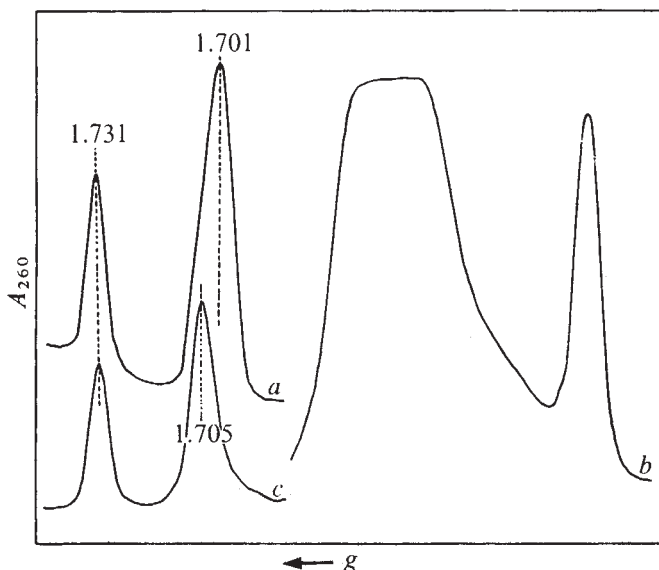


Fig. 2 a, Thermal denaturation of *S. sibirica* satellite DNA. Absorbance readings of T4 (○) and purified *S. sibirica* satellite DNA (●) in $0.25 \times \text{SSC}$ were taken at intervals in a Unicam SP 8000 spectrophotometer as the temperature control block was raised 1°C per 4 min. b, Renaturation of *S. sibirica* satellite DNA. Purified satellite DNA in $0.25 \times \text{SSC}$ was denatured at 100°C for 5 min, and then renatured at 70°C . The reciprocal of the remaining hyperchromicity ($1/(A_t - A_n)$, where $A_n = A_{260}$ of renatured DNA at time t) was plotted against time.

Fig. 1 Microdensitometer scans of ultraviolet photographs of total DNA from *Scilla sibirica*. DNA was prepared from young leaf tissue and analysed in a Model E analytical ultracentrifuge at 44,000 r.p.m. at 25°C for 20 h (ref. 19) a, $3 \mu\text{g}$ total DNA in $\text{CsCl} + 1 \mu\text{g}$ *Micrococcus lysodeikticus* marker DNA (1.731 g cm^{-3}); b, $20 \mu\text{g}$ of total DNA in Cs_2SO_4 with 0.3 M ratio of Ag^+ to DNA phosphate, $\text{pH } 8.0$; c, $3 \mu\text{g}$ of pure satellite DNA from the $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ gradient $+ 1 \mu\text{g}$ of *M. lysodeikticus* DNA in CsCl .



complexity to mouse satellite DNA and the fast component of melon satellite DNA¹¹.

Figure 3a–e shows photomicrographs of autoradiographs of cytological preparations made after hybridisation with complementary RNA (cRNA) transcribed *in vitro* from pure satellite DNA of *S. sibirica*. The grains are clearly localised at interphase (Fig. 3a) over the conspicuous chromocentres characteristic of the species^{10,12}. The label is 24 times more concentrated over the heterochromatin than the euchromatin, a factor which is not accounted for by the different density of DNA in heterochromatin, which is only 2.5–3 times^{13,14} more concentrated than the euchromatin. At metaphase (Fig. 3b–d) clusters of grains appear over restricted regions of the chromosomes. The longest homologous pair (1, Fig. 4b) and one of the shorter pairs (5) have grains restricted to the end of one arm. Another pair (2) is similar but in four of 18 such chromosomes examined grains were localised at both ends. The third pair contained relatively few grains, with localisation occurring in only four of 18 chromosomes examined. The fourth pair showed clear distal and interstitial grain clusters and the sixth showed localisation either at one end of the short arm or interstitially on the long arm, with about equal incidence. This may be explained by heterochromatic (H) segment heterozygosity¹⁵ or chromosome inversion. This distribution of satellite DNA corresponds closely with the H segments as revealed by cold treatment¹² (Fig. 4a). These H segments are stained by Geimsa¹⁶, and appear as bands of low fluorescence after treatment with quinacrine mustard or quinacrine¹⁷.

There are usually about twice as many grain clusters and H segments in the metaphase chromosomes as in the interphase

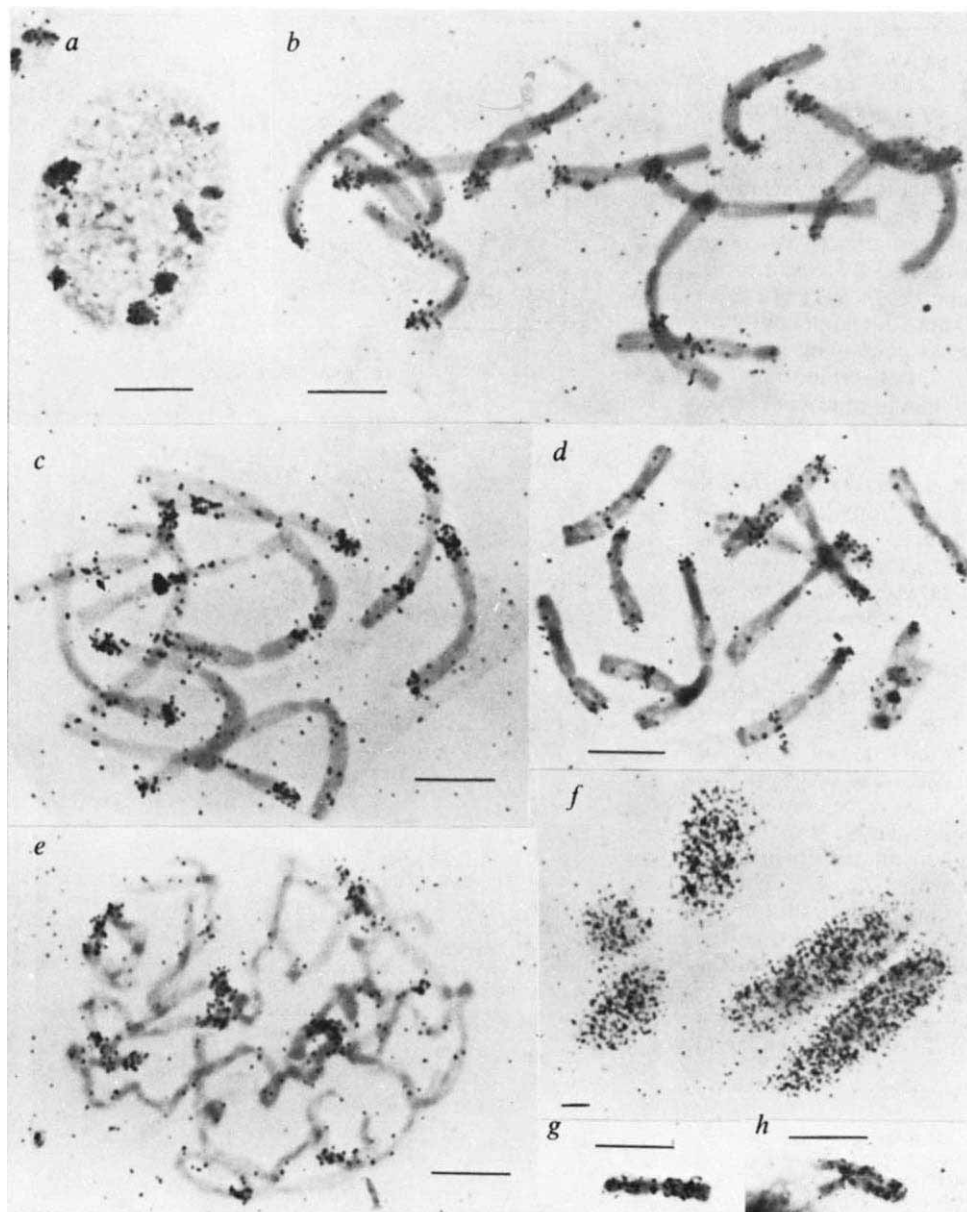
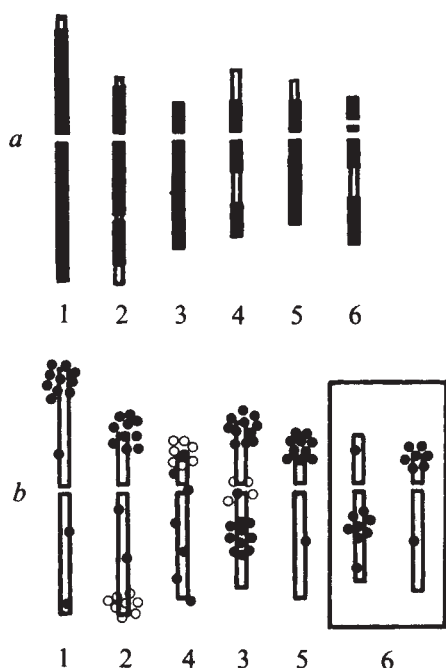


Fig. 3 *In situ* hybridisation of *S. sibirica* and *V. faba* satellite DNAs. RNA complementary to pure satellite DNA was transcribed *in vitro* using all four ^3H -nucleoside triphosphates²⁰. Colchicine-treated roots were fixed in 3:1 ethanol-acetic acid for 1 h at room temperature followed by further treatment with 45% acetic acid for 24 h. The root tips were squashed on to slides, the coverslips removed, and the preparations denatured in $0.1 \times \text{SSC}$ at 100°C for 0.5 min. After dehydration and air drying, the slides were hybridised with cRNA (specific activity 10^8 d.p.m. μg^{-1} , $2 \mu\text{g cm}^{-2}$) for 5.5 h at 75°C in $6 \times \text{SSC}$. The slides were washed in $2 \times \text{SSC}$, treated with RNase and extensively washed in $2 \times \text{SSC}$. After dehydration and drying the slides were coated with Ilford K2 photographic emulsion and exposed in a light-proof, desiccated box at 4°C for 5 weeks. The preparations were developed in Kodak D19b developer, fixed and washed before staining with Geimsa (pH 6.9) and mounting in Euparal. *a*, Interphase nucleus of *S. sibirica* showing localised labelling of the chromocentres. *b, c, d*, Metaphase cells of *S. sibirica* ($2n = 12$) showing mainly distal localisation of the hybridised cRNA. *e*, Prophase of *S. sibirica*. *f*, Interphase nuclei of *V. faba*. *g, h*, Individual chromosomes of *V. faba*. The bars represent 10 μm .



or prophase nuclei, (Fig. 3e), suggesting a link between repeated satellite sequences and somatic chromosome association and mechanics¹⁸.

In situ hybridisation of an $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ satellite DNA from *Vicia faba* is also shown in Fig. 3f-h. In this instance the satellite sequences appear to be more generally spread over the nuclei (Fig. 3f), and over the chromosomes (Fig. 3g,h). There is a suggestion that the ends of the chromosome arms are unlabelled and, in addition, certain proximal regions. It is interesting that, in contrast to *S. sibirica*, most of the *V. faba* chromosome shows low fluorescence after quinacrine treatment¹⁷, suggesting a possible correlation with the satellite DNA sequences.

We conclude that the satellite DNA of *S. sibirica* is highly localised at positions which closely correspond to those of the cold sensitive heterochromatin. Although this heterochromatin

Fig. 4 Idiograms of *S. sibirica* ($2n = 12$). *a*, The position of the heterochromatic (H) segments revealed by cold treatment at -12°C is indicated by open segments (after Baumann¹²). *b*, Summary of nine analyses of complete metaphase cells of *S. sibirica*, after *in situ* hybridisation, showing the positions of the grain clusters. Grain clusters always present (●), sometimes present (○). Chromosome 6 was present in our material in either of the alternative forms shown.

is in some ways different from the centromeric form observed in other species such as mouse, it also contains highly repeated satellite DNA sequences.

J. N. TIMMIS*
BARBARA DEUMLING
J. INGLE

Department of Botany,
University of Edinburgh,
Edinburgh EH9 3JH, UK

Received June 26; accepted July 25, 1975.

*Present address: Department of Botany, University College, Belfield, Dublin 4, Ireland.

- ¹ Arrighi, F. E., Mandell, M., Bergendahl, J., and Hsu, T. C., *Biochem. Genet.*, **4**, 367-376 (1970).
- ² Ingle, J., Pearson, G. G., and Sinclair, J., *Nature new Biol.*, **242**, 193-197 (1973).
- ³ Jones, K. W., *Nature*, **255**, 912-915 (1970).
- ⁴ Pardue, M. L., and Gall, J. G., *Science*, **168**, 1356-1358 (1970).
- ⁵ Jones, K. W., and Robertson, F. W., *Chromosoma*, **31**, 331-345 (1970).
- ⁶ Hennig, W., Hennig, I., and Stein, H., *Chromosoma*, **32**, 31-63 (1970).
- ⁷ Eckhardt, R. A., and Gall, J. G., *Chromosoma*, **32**, 407-427 (1971).
- ⁸ Jones, K. W., Prosser, J., Corneo, G., and Ginelli, E., *Chromosoma*, **42**, 445-451 (1973).
- ⁹ Ingle, J., Timmis, J. N., and Sinclair, J., *Pl. Physiol.*, **55**, 496-501 (1975).
- ¹⁰ La Cour, L. F., *Heredity*, **5**, 37-50 (1951).
- ¹¹ Sinclair, J., Wells, R., Deumling, B., and Ingle, J., *Biochem. J.*, **149**, 31-38 (1975).
- ¹² Baumann, T. W., *Expl Cell Res.*, **64**, 323-330 (1971).
- ¹³ Pearson, G. G., Timmis, J. N., and Ingle, J., *Chromosoma*, **45**, 281-294 (1974).
- ¹⁴ Fox, D. P., *Chromosoma*, **33**, 183-195 (1971).
- ¹⁵ Dyer, A. F., *Cytologia*, **29**, 155-170 (1964).
- ¹⁶ Schweizer, D., *Chromosoma*, **40**, 307-320 (1973).
- ¹⁷ Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Expl Cell Res.*, **58**, 128-140 (1969).
- ¹⁸ Walker, P. M. B., *Nature*, **229**, 306-308 (1971).
- ¹⁹ Wells, R., and Ingle, J., *Pl. Physiol.*, **46**, 178-179 (1970).
- ²⁰ Jones, K. W., in *New technique in biophysics and cell biology*, 1 (edit. by Pain, H. R., and Smith, B. J.), 29-66 (Wiley, New York and London, 1973).

Generation of discrete yeast DNA fragments by endonuclease RI

FINE structure genetic analysis has shown that the *ilv1* gene of yeast, *Saccharomyces cerevisiae*, is multifunctional¹⁻³. The *ilv1* gene product, threonine deaminase, shows catalytic activity, as well as participating in multivalent repression of other enzymes involved in isoleucine-valine biosynthetic pathways¹⁻³. A better understanding of the regulatory role of the *ilv1* gene product and other proteins, such as isoleucyl-tRNA synthetase, which in addition to the *ilv1* gene product seems to regulate *ilv2* and *ilv3* gene expression, could be obtained if a pure preparation of the various *ilv* genes were isolated in large quantities. One way of isolating the genes would be to use restriction enzymes to cleave yeast DNA which contains normal *ilv* genes and join the fragments to a bacterial plasmid. Because of the similarity of the isoleucine-valine pathways in yeast and *Escherichia coli*, the fused DNA could then be transformed into a strain of *E. coli* with a deletion in the particular *ilv* gene. Restriction enzyme-cleaved DNA from various sources has been amplified by growth in *E. coli*⁴⁻⁷. We have found that restriction enzyme *EcoRI* treatment of yeast DNA results in not only a reduction in the size of total DNA but also the generation of several distinct species of homogeneous size DNAs some of which are derived from ribosomal genes and others from mitochondrial genes.

DNA isolated from *S. cerevisiae* MAR-33 (ref. 8), although heterogeneous, was as large as 20×10^6 – 30×10^6 daltons. It migrates parallel to DNA of phage T3 and λ (molecular weight 23×10^6 and 31×10^6 , respectively) in 1% agarose. After treatment with *EcoRI* the yeast DNA profile was reduced in size but showed discrete bands. As Fig. 1 shows, at least ten distinct bands could be identified in a complete *EcoRI* digest of yeast DNA. Of these, the first and the last band was also present in untreated DNA. Thus, *EcoRI* treatment generated at least eight groups of homogeneously sized DNA fragments. More bands appeared in conditions of limited *EcoRI* digestion (Fig. 2). These seemed to be precursor, heavier DNA fragments that are found when *EcoRI* concentration is limiting (Fig. 2a) or the

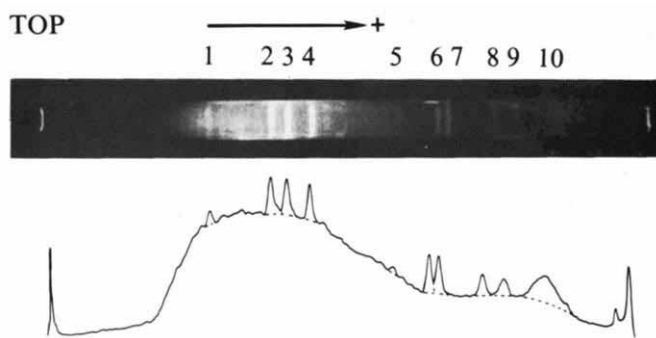


Fig. 1 *EcoRI*-yeast DNA fragments appearing as discrete bands. 100 μ g of yeast DNA was treated with 5 μ l of the restriction enzyme *EcoRI* for various intervals at 37 °C. Above is an electrophoresis pattern of 10 μ g of *EcoRI* DNA (3 h incubation with *EcoRI*) in 1% agarose gel containing 0.5 μ g ml⁻¹ of ethidium bromide and run at 4 mA per gel for 2.5 h at 24 °C. The negative photograph of the gel was run through a scanner and is presented below the picture of the gel.

digestion is carried out for a short interval, such as 2 min (Fig. 2b). These intermediates disappeared in about 6 min at 37 °C when *EcoRI* was not limiting.

The ten major bands were next compared with the sizes of *EcoRI*-cleaved fragments of phage λ DNA (Fig. 3). The designated molecular weights of these fragments—13.7, 4.7, 3.7, 3.5, 3.0 and 2.1×10^6 —show that all eight *EcoRI*-cleaved yeast DNA fragments are below 2×10^6 (Table 1). When the proportion of these DNA fragments was calculated from the areas under the peaks of the gel scan in Fig. 1 the minimum number of DNA copies in each band could be estimated (Table 1). The two DNA bands present in the untreated yeast DNA gave the two extreme values. While the heaviest band, 4.1×10^6 daltons, was present in at least 10 copies, the smallest band, 1.6×10^5 daltons, was present in at least 1,900 copies. Three *EcoRI*-cleaved yeast DNA bands were present in at least 100 copies (1.0×10^6 – 1.6×10^6

Fig. 2 Heavier intermediate bands generated by *EcoRI* treatment on yeast DNA. *a*, The experiments were performed as reported in the legend to Fig. 1 with 15 μ g of yeast DNA and various concentrations of *EcoRI*. Nearly 8 μ g of this DNA was run on a 0.6% agarose gel at 4 mA per gel for 2.5 h. *b*, The experimental condition is that described in legend to Fig. 1.

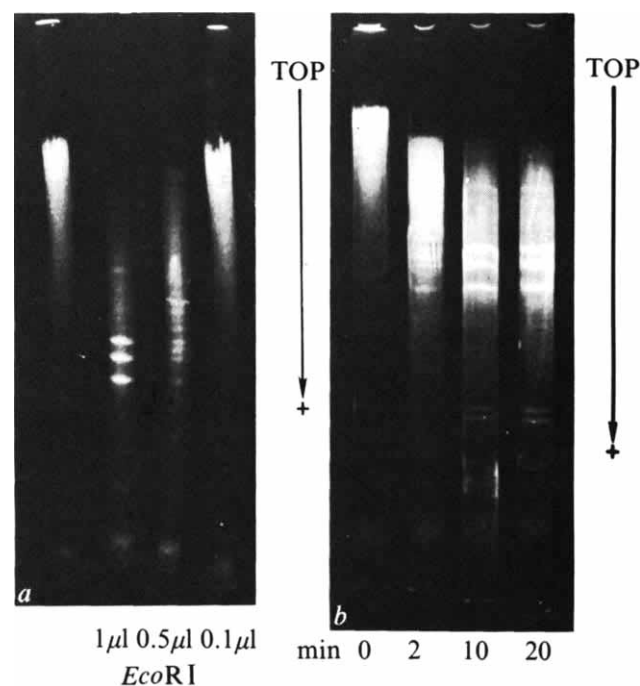


Table 1 Estimates of number of yeast DNA copies in bands appearing before and after *EcoRI* treatment

Band no.*	Molecular weight† × 10 ⁶ daltons	Proportion of total DNA§ (%)	No. of DNA copies¶
† 1	4.10	0.34	10
2	1.60	1.18	92
3	1.40	1.20	107
4	1.05	0.98	117
5	0.58	0.19	41
6	0.42	0.94	280
7	0.38	1.04	342
8	0.26	0.67	322
9	0.23	0.63	343
†10	0.16	2.33	1,880

* Appearance of bands in order of increasing mobility as shown in Fig. 1.

† These two bands are present before *EcoRI* treatment.

‡ Estimated from known molecular weights of λ DNA fragments in Fig. 3.

§ Obtained by measuring areas under peaks of the gel seen in Fig. 1.

¶ Calculated from a value of 1.25×10^{10} daltons for haploid yeast DNA.

daltons) and four in at least 325 copies (2×10^5 – 4×10^5 daltons). All ten bands combined constituted at least 10% of the total yeast DNA. Thus, the bulk of the *EcoRI*-cleaved yeast DNA was non-repetitive and heterogeneous in size.

Although the discrete bands of *EcoRI*-cleaved yeast DNA indicate that fragments of specific size were generated, they do not indicate whether they were of homologous sequence. There are nearly 140 copies of DNA for ribosomal RNA and 320–400 copies of DNA for transfer RNA in a haploid yeast¹⁰. Both 18S and 26S ribosomal RNA arise from a common precursor RNA molecule of 2.5×10^6 daltons (refs 11 and 12) which constitutes

a total ribosomal DNA of 400×10^6 – 700×10^6 daltons (ref. 13). Most of the 400×10^6 – 450×10^6 daltons of chromosome I codes for nearly 70% of the total ribosomal RNA^{14–16}. Bands 2, 3 and 4 of *EcoRI*-cleaved yeast DNA also constitute a total molecular weight of 420×10^6 daltons (Table 1). But then each of the 17–20 yeast chromosomal DNAs is of this molecular size—between 400×10^6 and 600×10^6 daltons (refs 13 and 17).

To determine which of the ten bands contained ribosomal DNA, 18S and 26S ribosomal RNA were purified from isolated ribosomal subunits and hybridised¹⁵ with DNA extracted from four segments of the complete gel and then from each of the *EcoRI*-generated bands. As Table 2a shows, the bulk of the hybridisation occurred in that segment of the gel which consisted of bands 2, 3 and 4 (Fig. 1). DNA isolated from bands 2 and 4 predominantly hybridised with 18S rRNA whereas the hybridisation with 26S rRNA was restricted to band 2 (Table 2b). Thus band 4 contains 18S rDNA. The hybridisation with band 2 indicates that this band either contains both 18S and 26S rDNAs or that the 18S RNA preparation contains some 26S RNA. In any event, a minimal assignment of 26S rDNA to DNA fragments in band number 2 and of 18S rDNA to band number 4 is possible.

Table 2 Hybridisation of yeast ribosomal RNA with *EcoRI*-generated yeast DNA fragments

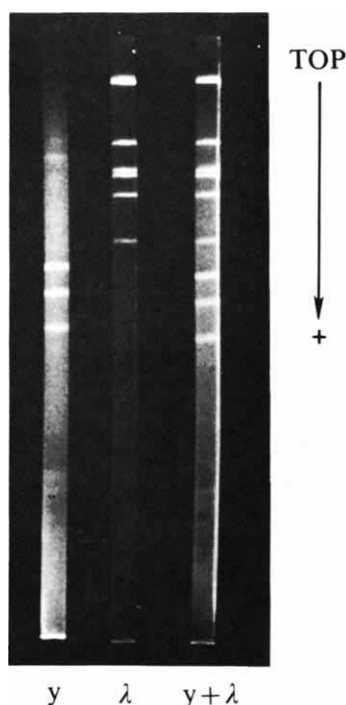
<i>EcoRI</i> -generated yeast DNA isolated from gel of Fig. 1	Hybridised with radioactive 18S 26S (c.p.m.)	
(a) Gel sections		
(i) From top to before band 2		
(ii) Band 2 to 4	211	135
(iii) Between band 4 and 6	1,053	903
(iv) From band 6 to 9	294	126
	328	212
(b) Band no.		
2	748	1,058
3	153	127
4	562	110
6 & 7	204	87
8 & 9	197	130

³H-ribosomal RNA (specific activity 0.3×10^6 c.p.m. μg^{-1}) was isolated from yeast cells grown in the presence of ³H-adenine. The 60S and 40S ribosomal subunits were separated and the two RNA species were prepared by sedimentation through SDS-sucrose gradients. DNA fragments were eluted from various portions of the gel shown in Fig. 1 and immobilised on nitrocellulose filters. Low temperature hybridisation in the presence of formamide was carried out on these DNA-loaded filters with either 18S ($4 \mu\text{g ml}^{-1}$) or 26S ($4.5 \mu\text{g ml}^{-1}$) rRNA by a method very similar to that of Finkelstein *et al.*¹⁵. Nonspecific binding of ³H-rRNA to blank filters gave a value of 15 c.p.m. above background and has been subtracted from the above values. The values reported are the averages of duplicates.

The contribution of mitochondrial DNA in generating these fragments could be quite significant. Analytical ultracentrifugation has shown that the yeast DNA preparation contains between 5 and 6% of mitochondrial DNA. This alone would not account for all the yeast DNA bands. Furthermore, yeast DNA preparations isolated after isopycnic centrifugation in CsCl showed similar bands after treatment with *EcoRI* all throughout the gradient profile. This suggests that the bands are not exclusively of mitochondrial DNA (light density)¹⁸ or ribosomal DNA (heavy density)^{18–20} in origin. Bands 6 to 9 could be mitochondrial, however, since they are absent when yeast nuclear DNA separated from mitochondrial DNA by CsCl equilibrium centrifugation is subjected to *EcoRI* digestion and applied to the 1% agarose gel.

In conclusion, most yeast DNA fragments generated by *EcoRI* treatment are not highly repetitive, although several classes of homogeneously sized DNA species are found. Some of the

Fig. 3 Size relation of *EcoRI*-yeast DNA to *EcoRI*-phage λ DNA. 10 μg of bacteriophage λ DNA and 20 μg of yeast DNA were digested repeatedly with *EcoRI*. 2 to 4 μg of these *EcoRI* treated DNA individually and combined were run in a 1% agarose gel at 0.6 mA per gel for 17 h in the absence of ethidium bromide. The gels were soaked for 30 min with $5 \mu\text{g ml}^{-1}$ of ethidium bromide before photography.



enriched DNA species seem to be mitochondrial while two size classes contain ribosomal DNA sequences. The easy availability of DNA sequences such as those for the ribosomal genes should enhance the development of the methodology for the isolation of the *ilv* structural genes.

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KAMALENDU NATH
ARTHUR P. BOLLON

Department of Biochemistry,
University of Texas Health Science Center at Dallas,
Dallas, Texas 75235

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- ¹ Bollon, A. P., *Nature*, **250**, 630–634 (1974).
- ² Bollon, A. P., and Magee, P. T., *J. Bact.*, **113**, 1333–1344 (1973).
- ³ Bollon, A. P., and Magee, P. T., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2169–2173 (1971).
- ⁴ Cohen, S. N., Chang, A. C. Y., Boyer, H. W., and Helling, R. B., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3240–3244 (1973).
- ⁵ Chang, A. C. Y., and Cohen, S. N., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1030–1034 (1974).
- ⁶ Morrow, J. F., et al., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743–1747 (1974).
- ⁷ Hershfield, V., Boyer, H. W., Yanofsky, C., Lovett, M. A., and Helsinki, D. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3455–3459 (1974).
- ⁸ Betz, J. L., Hereford, L. M., and Magee, P. T., *Biochemistry*, **10**, 1818–1824 (1971).
- ⁹ Thomas, M., and Davis, R. W., *J. molec. Biol.*, **91**, 315–328 (1975).
- ¹⁰ Schweizer, E., MacKeechne, C., and Halvorson, H. O., *J. molec. Biol.*, **40**, 261–277 (1969).
- ¹¹ Taber, R. L., and Vincent, W. S., *Biochim. biophys. Acta*, **186**, 317–325 (1969).
- ¹² Udem, S. A., and Warner, J. R., *J. molec. Biol.*, **65**, 227–240 (1972).
- ¹³ Blamire, J., Cryer, D. R., Finkelstein, D. B., and Marmur, J., *J. molec. Biol.*, **67**, 11–24 (1972).
- ¹⁴ Oyen, T. B., *FEBS Lett.*, **30**, 53–56 (1973).
- ¹⁵ Finkelstein, D. B., Blamire, J., and Marmur, J., *Nature new Biol.*, **240**, 279–281 (1972).
- ¹⁶ Kaback, D. B., Bhargava, M. M., and Halvorson, H. O., *J. molec. Biol.*, **79**, 735–739 (1973).
- ¹⁷ Petes, T. D., and Fangman, W. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1188–1191 (1972).
- ¹⁸ Goldberg, S., Oyen, T., Idriss, J. M., and Halvorson, H. O., *Molec. gen. Gen.*, **116**, 139–157 (1972).
- ¹⁹ Cramer, J. H., Bhargava, M. M., and Halvorson, H. O., *J. molec. Biol.*, **71**, 11–20 (1972).
- ²⁰ Retel, J., and Planta, R. J., *Biochim. biophys. Acta*, **281**, 299–309 (1973).

Determination of 5'-triphosphate terminus of 16S ribosomal RNA precursor

THE genes for the 23S and 16S rRNAs are physically linked¹. Using the drugs rifampicin and actinomycin D, it has been shown that the 23S and 16S rRNA cistrons are co-transcribed and the promoter is proximal to 16S rRNA cistron^{2,3}. Recent work with an *Escherichia coli* strain deficient in RNase III has provided further evidence for co-transcription, showing the presence of a long polycistronic RNA which could be cleaved to the immediate precursors of 23S and 16S rRNAs when treated with RNase III *in vitro*⁴. We have now studied the transcription of rRNA in *E. coli* using γ -³²P-labelled triphosphates and have shown that the 16S rRNA precursor in our preparation has a 5'-pppA end and that in our system it is neither cleaved nor replaced by 5'-pA as observed by Takanami⁵. We have not found any detectable 5'- γ -³²P-triphosphate end in the 23S rRNA species. This observation further verifies directly that both the 16S and 23S rRNAs belong to one transcriptional unit in the following order: promoter–16S–23S.

RNA transcription proceeds in the 5'→3' direction and the first nucleotide at the 5' end has so far been found to be a purine⁶, either ATP or GTP. We used γ -³²P-ATP and γ -³²P-GTP to study the initiation of rRNA transcription in *E. coli* spheroplasts. The presence of radioactivity in the nucleotide triphosphates of the RNA hydrolysate would represent incorporation of the label at the 5' end of the RNA. The advantage of using *E. coli* spheroplasts is that there is insignificant

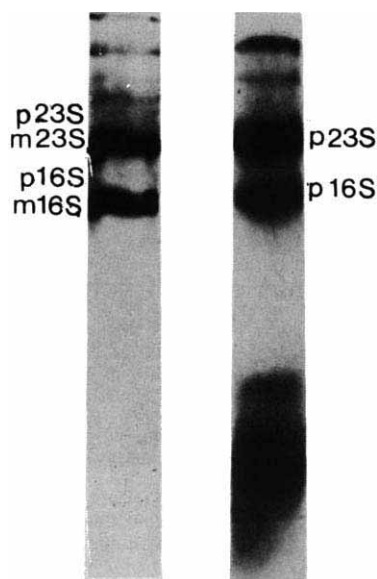


Fig. 1 *E. coli* CP78 (*his*⁻, *leu*⁻, *thr*⁻, *arg*⁻, *thi*⁻, *rel*⁺) was grown in 20 ml minimal M9 medium until absorbance reached $0.1 (2 \times 10^8 \text{ cells ml}^{-1})$. The culture was then spun down at 6,000 r.p.m. for 10 min at room temperature. The pellet was resuspended and shaken in 20 ml of a plasmolysing buffer containing 10^{-3} M EDTA , 0.12 M Tris , $\text{pH } 7.8$ for 3 min. This rendered the cells permeable to nucleotide triphosphates for no less than 15 min. The cells were then poured into 20 ml pre-warmed M9 minimal medium containing $0.5 \text{ mCi } \gamma$ -³²P-ATP or $0.5 \text{ mCi } \gamma$ -³²P-GTP (New England Nuclear, specific activity containing less than 0.1% of α -³²P-ATP and α -³²P-GTP as well as orthophosphate. γ -³²P-GTP has a specific activity of $0.0552 \text{ mCi mol}^{-1}$ and γ -³²P-ATP of $0.0316 \text{ mCi mol}^{-1}$). This labelling procedure was terminated after 15 min by pouring the culture into 5 ml frozen M9 buffer, and immediately spun down at 4°C . RNA extraction was then carried out by resuspending the pellet in 25% sucrose, 0.01 M Tris , $\text{pH } 7.2$, and then following the hot phenol technique described in ref. 8. The RNA extract was precipitated by adding 0.1 ml ml^{-1} of 3 M sodium acetate, and 2 volumes of cold ethanol, and left overnight at -20°C . The precipitate was spun at $10,000 \text{ r.p.m.}$ for 20 min, and resuspended in 1 ml of 0.01 M sodium acetate buffer, $\text{pH } 5.1$. The RNA (total) was analysed through a 5 cm , 1.2% polyacrylamide-agarose gel, and electrophoresed at 5 mA and 50 V for 1.75 h . The gels were sliced and exposed to X-ray sensitive films and developed.

background due to pyrophosphate exchange and little breakdown of triphosphates to monophosphates. Thus incorporation of nucleotides with phosphate labelled at the α -position is negligible.

In this system, the transcription is qualitatively normal, as evidenced by our separate studies on ³H-UTP uptake and titrations of *lac* or *gal* mRNA. The rate of RNA synthesis is, however, reduced to 30% . This was also observed by others⁷. The nucleases responsible for processing the 16S and 23S precursors in this system become very inefficient, as shown in autoradiograms of RNA isolated from spheroplasts and subjected to polyacrylamide gel electrophoresis (Fig. 1). As Fig. 1 shows, the autoradiogram of the normal rRNAs in *E. coli* has four bands. From top to bottom of the gel they are the precursor 23S (p23S), matured 23S m23S, precursor 16S (p16S) and mature 16S (m16S) RNA. As for the rRNAs from the *E. coli* spheroplasts only the 23S and 16S precursors are seen.

RNAs labelled with γ -³²P-nucleotide triphosphate were isolated from *E. coli* spheroplasts. They were fractionated into p23S and p16S by sucrose gradient and digested by alkali treatment (see footnote of Table 1). The hydrolysates from p23 and p16 were then spotted on thin-layer chromatographic sheets

Table 1 Radioactivity from the alkaline hydrolysates of rRNAs isolated from *E. coli* CP78 spheroplast

³² P-labelled purine nucleotides	Radioactivity (c.p.m.) obtained from TLC spots with rRNA samples*	
	p16S†	p23S†
³² pppAp	367	0
³² pppGp	0	0
AMP ³²	187	386
GMP ³²	146	337

* γ -³²P-labelled ATP or GTP was used to label RNA isolated from *E. coli* CP78 spheroplasts. The RNA extract (for method see legend under Fig. 1) was subjected to a linear 5–20% sucrose gradient centrifugation at pH 5.1, using SW 40 rotor and Beckman ultracentrifuge L5-40 at 4 °C and centrifuging for 12 h at 25,000 r.p.m. The fractions containing p23S and p16S RNAs were collected and their specific activities determined by A_{260} (410 and 487 c.p.m. μg^{-1} , respectively). These two species of rRNA were first dialysed in distilled water for 6 h to eliminate sucrose, and then hydrolysed separately by treating with 0.3 M KOH at 37 °C for 16 h. The hydrolysate contains nucleotide tetraphosphates and monophosphates⁶. The hydrolysates were then neutralised by adding appropriate volumes of 1 M perchloric acid. The precipitate, potassium perchlorate, was pelleted by centrifugation at 12,000 r.p.m. for 20 min in a Sorvall RC2B centrifuge at 4 °C. Fractions of the supernatant (600 c.p.m. each or approximately 1.5 μg) were spotted on and analysed by thin-layer chromatography using plastic backed sheet obtained from Macherey-Nagel, Polygram cel 300 PEI. Non-radioactive ATP AMP, GTP and GMP were also spotted on the same sheet as reference markers. The chromatographic sheet was run in 1 M LiCl, 1 M acetic acid for 2.5 h at 4 °C. The various spots could be identified by exposing the sheet under a mineral ultraviolet lamp. The tetraphosphates and monophosphates spots were cut out carefully, dried at room temperature for 2 h and then counted on a Packard scintillation counter (Model 3375, Tri-Carb) in toluene-based Omnifluor.

†p16S and p23S refer to precursors of 16S and 23S rRNA species.

and separated. The results are tabulated in Table 1. There is a conspicuous tetraphosphate spot for p16S RNA hydrolysate that is not detectable in the case of p23S rRNA hydrolysate. Therefore, for the p23S rRNA hydrolysate, the region corresponding to the tetraphosphate spot was located by adjusting with the 16S rRNA spot by eye estimation, and a considerable area of the TLC around the region was cut. Lines 1 and 3 of column 4 (Table 1) represent the radioactivity (c.p.m.) of the tetraphosphate spots for p16S (precursor of 16S rRNA) and p23S (precursor of 23S rRNA) rRNA hydrolysates, which are 367 c.p.m. and 0 c.p.m. respectively. The first and third line in Table 1, column 5 are the radioactivity due to XM³²P spots of p16S and p23S hydrolysates.

Table 1 indicates that there is a breakdown of the γ -³²P-triphosphate label, but the extent is not very high. Considering the specific activity of γ -³²P-ATP (2.5 $\mu\text{Ci ml}^{-1}$) used for labelling rRNAs and the fact that only one 5'-³²P-labelled ATP would appear on the 5' end of each p16S RNA molecule the amount of ³²P-monophosphate, incorporated along the length of p16S or p23S rRNA is calculated as less than 0.0001% of input radioactivity. Incorporation of the labelled phosphorus into the entire length of the rRNA molecules is presumably the reason for the presence of approximately double the number of c.p.m. in the monophosphate spots of p23S compared with that of p16S RNA. γ -³²P-nucleotide triphosphates were incorporated into the p16S rRNA but not into the p23S rRNA. Because the first nucleotide incorporated always retains its triphosphate ends, we can minimise the possibility of pyrophosphate exchange into the triphosphate position. Therefore any radioactivity present in the tetraphosphate spots would be the γ -labelled ³²P-nucleotide triphosphates.

To verify which of the two purines (γ -³²P-ATP or γ -³²P-GTP) was incorporated as the first nucleotide, similar experimental procedures were repeated using only γ -³²P-GTP. Results are tabulated in Table 1, lines 2 and 4. Tetraphosphate spots of both p16S and p23S RNA hydrolysates contained no detectable radioactivity. This suggests that GTP was not the

initiating nucleotide triphosphate and therefore ATP must be the first nucleotide incorporated.

We therefore conclude, since the p16S rRNA hydrolysate contained ³²P-radioactivity in its tetraphosphate spot and p23S rRNA did not, that: (1) initiation of rRNA transcription can only take place in the 16S rRNA gene, (2) ATP is the initiating nucleotide, and (3) transcription initiating at 16S rRNA proceeds towards 23S rRNA without further reinitiation.

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PATRICK W. TANG
ARABINDA GUHA

Microbiology Department,
University of Toronto,
Erindale College,
Mississauga, Ontario, Canada

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- 1 Doolittle, W. F., and Pace, N. R., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1786 (1971).
- 2 Doolittle, W. F., and Pace, N. R., *Nature*, **228**, 125 (1970).
- 3 Bleyman, M., Kondo, M., Hecht, N., and Woese, C. R., *J. Bact.*, **99**, 535 (1969).
- 4 Nikolav, N., Silengo, L., and Schlessinger, D., *J. biol. Chem.*, **248**, 7967 (1973).
- 5 Takanami, M., *J. molec. Biol.*, **23**, 135 (1967).
- 6 Maitra, V., and Hurwitz, J., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 815 (1975).
- 7 Bea-Hamida, F., and Gros, F., *Biochimie*, **53**, 71 (1971).
- 8 Guha, A., Saturen, Y., and Szybalski, W., *J. molec. Biol.*, **56**, 53 (1971).
- 9 Jorgensen, S. E., Buch, L. B., and Nierlich, D. P., *Science*, **164**, 1067 (1969).

Corrigenda

In the article "Collimation of auroral particles of time-varying acceleration" by D. S. Hall and D. A. Bryant (*Nature*, **251**, 402; 1974) there is an error in equation (10). The denominator should read $\sqrt{2(\sigma/E_0)}$ and not as printed. Also the value of A in the legend to Fig. 1 should read $9.1 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ keV}^{-1}$ and not as printed.

In the article "Oxidation of nickel by nitric oxide as a new strong oxidant" by Y. Takasu, Y. Matsuda, S.-I. Maru and N. Hayashi (*Nature*, **255**, 544; 1975) there are several errors in the figures and legends. On the ordinate of Figs 2 and 3, for K_1 read K_1' . The legends to Figs 2 and 3 should read:

Fig. 2 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and temperature ($P_{\text{NO}} = 10 \text{ mmHg}$). $E = 17.8 \text{ kcalorie}$.

Fig. 3 Relationship between K_1' ($\text{g cm}^{-2} \text{ s}^{-1}$) and the pressure of nitric oxide (700 °C). $n = 0.74$.

Errata

In the article "Excision of thymine dimers from specifically incised DNA by extracts of xeroderma pigmentosum cells" by K. Cook, E. C. Friedberg and J. E. Cleaver (*Nature*, **256**, 235; 1975) the following corrections should be made. In line 3 of the legend to Table 1, for 50–100 erg mm^{-2} read 5–10 J m^{-2} . The list of authors should include as the last author H. Slor, Department of Human Genetics, University of Tel Aviv, Israel. The asterisk after J. E. Cleaver should indicate that his address is the Laboratory of Radiobiology, University of California School of Medicine, San Francisco, California.

In the article "Pathogenicity and cerato-ulmin production in *Ceratomyces ulmi*" by S. Takai (*Nature*, **252**, 124; 1974) there is an error in the second sentence of paragraph 6. For (120 mg ml^{-1}) read (120 $\mu\text{g ml}^{-1}$).

matters arising

Formaldehyde polymers in interstellar space

THE suggestion¹ that polyoxymethylene (POM) crystals exist in interstellar space must be reviewed in terms of the thermodynamics of polymer-monomer equilibria². The formaldehyde-POM equilibrium is notoriously mobile³, unless the polymer is end-capped⁴. This treatment is applied to commercial samples such as those whose infrared spectra were reported by Tadokoro *et al.*⁵, which provided data for the argument¹ for POM in space.

The partial pressure of formaldehyde in equilibrium with POM has been determined over a small temperature range⁶, and, by extrapolation, the partial pressure at 20 K (the temperature at which the polymer is supposed to form¹) is equivalent to $2 \times 10^{-14.3}$ molecules cm^{-3} . Chemical thermodynamics thus supports the hypothesis that the polymer exists in some regions of space: a mechanism for initiating the polymerisation process on the surface of mineral particles may be provided by ionising radiation, though the rate of propagation must be extremely small at such a temperature.

At 445 K, the temperature assumed for the dust in the Trapezium nebula¹, the equilibrium partial pressure of formaldehyde above POM, is more than 1 atmosphere, which is greatly in excess of any likely nebulous condition. Only if there were a means for end-capping interstellar POM would it exist at 445 K, and the survival time of such material in the ionising radiation of an H II region would be short. It is, therefore, doubtful that the infrared emission from the Trapezium nebula is from a POM source at that temperature.

It is interesting to note that the concentration of formaldehyde molecules in the Trapezium nebula seems to be greater where ionised atomic material is most dense⁷, and it is possible to postulate a role for the formaldehyde-POM equilibrium in the globular model⁸ of the core of the nebula, assuming that the temperature and formaldehyde molecule concentrations were suitable.

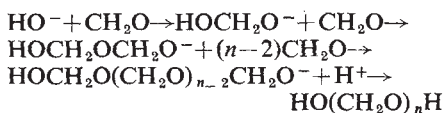
A. H. FAWCETT

Department of Chemistry,
The Queen's University of Belfast, UK

- ¹ Wickramasinghe, N. C., *Nature*, **252**, 462 (1974).
- ² Dainton, F. S., and Ivin, K. J., *Q. Rev. chem. Soc.*, **12**, 61 (1958).
- ³ Staudinger, H., *Helv. chim. Acta*, **8**, 67 (1925).
- ⁴ Barker, S. J., and Price, M. B., *Polyacetals* (The Plastics Institute, 1970).
- ⁵ Tadokoro, H., Kobayashi, M., Kawaguchi, Y., Kobayashi, A., and Murahashi, S., *J. chem. Phys.*, **38**, 703 (1963).
- ⁶ Busfield, W. K., and Merigold, D., *Makromolekul. Chem.*, **138**, 65 (1970).
- ⁷ Eliot, K. H., and Meaburn, J., *Nature phys. Sci.*, **244**, 69 (1973).
- ⁸ Dyson, J. E., *Astrophys. Space Sci.*, **1**, 388 (1968); *ibid.*, **2**, 461 (1968).

WICKRAMASINGHE AND SANTHANAN
REPLY—Preliminary remarks on the end-capping of formaldehyde polymers have already been made in the context of the formation of interstellar POM¹. The vapour pressure data for such stabilised polymers (for example, resins consisting mainly of $(\text{H}_2\text{CO})_n$) do not seem to be readily available. The extrapolations of vapour pressure^{2,3} data discussed by Fawcett⁴ relate to unstabilised POM. For a gas kinetic temperature of 100 K, as may be appropriate to an H I region, the number density of gaseous H_2CO molecules in equilibrium with unstabilised POM reaches the observed value $\sim 10^{-8} \text{ cm}^{-3}$ at a particle temperature, T , $\sim 115 \text{ K}$. As that temperature is less than the temperature of most grains, there can be no doubt about the stability of POM even if it were not suitably end-capped in normal interstellar conditions.

We consider it unlikely, however, that interstellar polyformaldehyde will not be end-capped. The formation of POM mantles or whiskers is expected to occur mainly in molecular clouds in the presence of considerable abundances of OH, H_2O and other molecules including CH_3OH . The H_2CO polymerisation could be initiated by the well established process of anionic polymerisation⁵. For example, HO^- from interstellar water molecules may have a crucial role:



End-capping which would ensure maximum stability may be achieved by a similar interaction with interstellar methanol (CH_3OH) molecules.

The polymerisation of interstellar formaldehyde could be initiated and

end-capped by this or any other process involving anionic attack. Suitably end-capped formaldehyde polymers (for example, CH_2O resins) are highly stable at room temperature. Such crystalline polymers are expected to have sufficiently low vapour pressures to ensure their stability in H II regions up to temperatures, T , of about 400–500 K, or even higher. Particles heated to temperatures T , of more than 300 K could account for the observed 8–12 band in the Trapezium nebula^{5,6}.

University College, Cardiff;
Tata Institute of Fundamental Research,
Bombay, India

- ¹ Wickramasinghe, N. C., *Mon. Not. R. astr. Soc.*, **170**, 11 (1975).
- ² Dainton, F. S., and Ivin, K. J., *Q. Rev. chem. Soc.*, **12**, 61 (1958).
- ³ Busfield, W. K., and Merigold, D., *Makromolekul. Chem.*, **138**, 65 (1970).
- ⁴ Fawcett, A. W., *Nature*, **257**, 159 (1975).
- ⁵ Ravve, A., *Organic Chemistry of Macromolecules*, 91 (Dekker, New York, 1947).
- ⁶ Wickramasinghe, N. C., *Nature*, **252**, 462 (1974).

Longitudinal photons in the fireball

BYRNE AND BURMAN have discussed the significance of astrophysical data for the problem of the photon mass¹. A question might arise concerning the possible presence of longitudinal photons in the microwave background were the photon mass not zero. In true thermal equilibrium, the longitudinal modes of the field would have to be populated to the same extent as the transverse modes. Bass and Schrödinger concluded some time ago that millions of years would be required for an appreciable fraction of longitudinal photons to build up in any terrestrial situation². Such time intervals, however, are available when dealing with cosmology.

The probabilities for the emission of longitudinal and transverse vector mesons with wave number k in a given process are in the ratio $m^2 c^2 / (\hbar^2 k^2 + m^2 c^2)$, where m is the meson rest mass³. When $\hbar k \gg mc$, this is approximately $(\lambda/\lambda_c)^2$, where λ is the wavelength and $\lambda_c = \hbar/mc$. If the mean free path of the photon is L , the number of collisions per second is c/L , and the fraction of transverse photons converted

into longitudinal photons every second will be $(c/L)(\lambda/\lambda_c^2) \equiv 1/\tau$. Also $1/L \approx n\sigma$, where n is the density of scatterers and σ the scattering cross section. We are concerned with the photons of the fireball moving through hydrogen, and can assume σ to be the cross section for Rayleigh scattering: $\sigma \approx a^2\lambda_b^4/\lambda^4$, where a is the classical electron radius and λ_b is the wavelength corresponding to the binding energy for hydrogen, about 10^{-5} cm. Both λ and n will change because of the expansion of the Universe: $\lambda = S\lambda_0$ and $n = n_0/S^3$, where λ_0 and n_0 are the quantities at epoch t_0 and S is the cosmological scale factor, chosen so that $S_0 = 1$. That gives:

$$\tau \approx S^5 \lambda_0^2 \lambda_c^2 / c n_0 a^2 \lambda_b^4$$

The factor τ depends strongly on S , which is now of the order of 1,000 if the initial epoch is that of the decoupling of the fireball radiation and matter; it is smallest when $S = 1$, $n_0 \approx 10^4$ atom cm^{-3} and $\lambda_0 \approx 10^{-4}$ cm. If $m = 10^{-52}$ g (ref. 4) then $\tau \approx 10^{44}$ yr. Thus, the fraction of longitudinal photons in the field after even many times 10^{10} yr is negligible. Even though long times are available, the free paths of photons are too long for sufficient scattering events to occur.

GEORGE L. MURPHY

Department of Physics,
Westminster College,
New Wilmington,
Pennsylvania 16142

¹ Byrne, J. C., and Burman, R. R., *Nature*, 253, 27 (1975).

² Bass, L., and Schrödinger, E., *Proc. R. Soc.*, A232, 1-6 (1955).

³ Coester, F., *Phys. Rev.*, 83, 798-800 (1951).

⁴ Byrne, J. C., and Burman, R. R., *J. Phys.*, A6, L12-L14 (1973).

Do epicentres migrate on the San Andreas Fault?

WOOD AND ALLEN¹ have suggested that seismicity on a portion of the San Andreas Fault can be used in support of a model of recurring migration of epicentres. They considered 27 earthquakes of magnitude, $M \geq 5$ within 30 km of the fault between 35.5°N and 38.5°N which occurred between 1930 and 1972. Five parallel line segments are drawn through the time-latitude plot of 27 points; the common slope of the lines is presumed to give the velocity of migration of earthquakes. Several of these events correlate with one another; any line in the neighbourhood of events that are close together in latitude and time will provide a satisfactory fit.

Obvious clustered events are the quadruplet (1,2,3,4) and the pairs (15,16; 5,6; 19,20; and 25,26) (Table 1 of Wood and Allen). Probably, other correlated events could be found were a different model used. Further, event 13 seems not to have been used in the fit.

Thus, there are 19 or fewer independent events in the data set. If a model is used in which the slopes of the line segments are fixed, then there are 19 or fewer degrees of freedom in the data, which are used to fit the intercepts of the segments.

The number of degrees of freedom in the model used by Wood and Allen may be about 15 in addition to the common slope: that is, the intercepts and end points of each of the five lines. The number of degrees of freedom in the end points is a function of the length of the segments and is difficult to evaluate. Thus, the number of degrees of freedom in the model is roughly equal to the number of degrees of freedom in the data, and we conclude that there are inadequate data to support the parametric complexity of the migration model. We believe that the apparent migration of epicentres along part of the San Andreas Fault is an artefact of Wood and Allen's model.

Wood and Allen forecast an earthquake with $M \geq 5$ in a restricted time-space interval along the San Andreas Fault. Though the complexity of the model makes it easy to give qualitative predictions, quantitative predictions in terms of probabilities remain difficult. Wood and Allen have avoided the question of estimating the risk that is: the probable number of shocks that will occur in the time-space region identified as dangerous. We can calculate the risk on the basis of a simpler model involving a considerably smaller number of degrees of freedom. Suppose that earthquakes are Poissonian between 36.75° and 37.05°N . Seven earthquakes are then found in the $25 \text{ yr} \times 33 \text{ km}$ time-space region; the area identified as dangerous by Wood and Allen is about $6 \text{ yr} \times 17 \text{ km}$. Thus, 0.86 ± 0.31 shocks can be expected in the defined time-space interval. Dropping the constraint that the earthquake catalogue has a space-time envelope, and assuming instead that shocks occurred randomly between 1930 and 1972, then 0.50 ± 0.19 shocks with magnitudes of more than five will occur in the smaller time-space interval.

Y. Y. KAGAN
L. KNOPOFF

Institute of Geophysics and Planetary
Physics,
University of California,
Los Angeles, California 90024

¹ Wood, M. D., and Allen, S. S., *Nature*, 244, 213 (1973).

Competition and species abundance

HEBERT *et al.*¹ used samples of the aerial population of Macrolepidoptera, collected at light in Ontario, Canada, to advance an argument in competitive population dynamics. Although not entirely explicit, it seems to run as follows. Given the "generally conceded" initial premise that interspecific competition is greatest between closely related sympatric species, the hypothesis that species abundance is controlled by level of competition can be tested by the relationship between mean species' population density and their taxonomic distance. In other words, in a sample from a multispecies population, the mean number of individuals per species in each family (N_s) is expected to be negatively correlated with the number of species in that family (S_f); making the provisional assumption that the relationship is causal.

The general conclusion reached by Hebert *et al.*¹ from their evidence of an inverse relation between family size and the abundance of species, was as expected; "family differences . . . are due to competitive interactions." The experiment raises questions at all levels, technical, analytical, interpretative and logical and, because the issue is a central one, we will take these up in more detail elsewhere. Our present purpose is to show that results like these are not invariably obtained from samples of this kind and, therefore, the conclusion as stated is not general.

The data in Table 1 are sums of seven replicate samples, each one a single year's

Table 1 Species abundance and mean no. of individuals per species in each family at two selected sites in Britain

	Malham		Bangor	
	S_f	N_s	S_f	N_s
Noctuidae	86	69.03	79	10.00
Geometridae	75	90.51	103	27.15
Arctiidae	5	4.60	6	4.00
Notodontidae	4	9.75	2	3.50
Sphingidae	1	6.00	2	1.50
Other families*	1.6	16.63	2	2.33

* Five at Malham and six at Bangor.

accumulation of nightly subsamples of Macrolepidoptera collected at light, from Malham in northern England² and Bangor in North Wales, as part of the Rothamsted Insect Survey³ during 1966-72. These sites were selected from a much larger series because, in these particular instances, the relation between N_s and S_f is diametrically opposed to that given by Hebert *et al.*; the correlation is positive, not negative.

The use of selective samples to investi-

gate population properties of the kind involved here is not new⁴ and their validity is currently being investigated^{5,6}. So far, we can find no objection in principle to the technique, although the selection mechanism used to obtain a sample is obviously confounded with some behavioural properties, thus invalidating its use for certain purposes. In the present instance, the difference between our results and those of Hebert does not seem to be accountable to such artefacts and we do not question the validity of either set of data. Consequently, one must either reverse Hebert's "generally conceded" initial premise, or, following their logic, regard our result as establishing that abundance is positively dependent on competition in the Malham and Bangor communities.

As Hebert *et al.*¹ point out, we are not here concerned with replacement competition but with the diffuse competition⁷, resulting from common environmental interests⁴ and mortality factors, in which the theory of island biogeography⁸ has generated much interest. While we have good reason to regard neither experiment as conclusive, because both are partial, our positive result might seem to indicate the negative competition allowed for in Hutchinson's original niche concept⁹.

L. R. TAYLOR
I. P. WOIWOD

Entomology Department,
Rothamsted Experimental Station,
Harpenden, Hertfordshire, UK

- ¹ Hebert, P. D. N., Ward, P. S., and Harmsen, R., *Nature*, **252**, 389-391 (1974).
- ² Kempton, R. A., and Taylor, L. R., *J. anim. Ecol.*, **43**, 381-399 (1974).
- ³ Taylor, L. R., *Rept Rothamsted expl. Stn 1973 Pt 2*, 202-239, (1974).
- ⁴ Williams, C. B., *Patterns in the Balance of Nature*, (Academic, London, 1964).
- ⁵ Taylor, L. R., and Brown, E. S., *Bull. ent. Res.*, **62**, 91-112 (1972).
- ⁶ Taylor, L. R., and French, R. A., *Bull. ent. Res.*, **63**, 583-594 (1974).
- ⁷ MacArthur, R. H., Diamond, J. M., and Karr, J. R., *Ecology*, **53**, 330-342 (1972).
- ⁸ MacArthur, R. H., and Wilson, E. O., *The Theory of Island Biogeography*, (Princeton University Press, Princeton, 1967).
- ⁹ Hutchinson, G. E., *Symp. quant. Biol.*, **22**, 415-427 (1957).

HEBERT ET AL. REPLY—Since diffuse competition in Lepidoptera, as we view it, is the result of long term coevolution among lepidopterans, their food plants, predators and parasites, we would not expect these interactions to be strong in highly disturbed communities. The importance of analysing pristine communities in synecological studies of this sort has been recognised¹. The drastic alteration of the original vegetation in Great Britain probably precludes such studies. Ter-

borgh¹, for example, worked in a rain-forest wilderness area of 20,000 km², whereas the least disturbed site in the Rothamsted Insect Survey consisted of 3.2 acres of 80-yr-old regrowth². Our main study area (Perth Road, Ontario)³ was somewhere between these extremes; within a 1-km radius of the collecting site there was little intrusion of introduced plants and forest composition approached a climax condition for the area. Beyond this distance most of the landscape was similar within a radius of 10 km, although scattered farms were present. Similarly, at our Chaffey's Locks site, much of the original vegetation remains intact.

With regard to the effect of environmental disturbance on abundance patterns

Table 1 Species abundance at Glenburnie, Ontario

	No. of species	No. of individuals per species
Noctuidae	248	51.85
Geometridae	86	25.38
Notodontidae	28	7.68
Arctiidae	27	42.15
Sphingidae	17	10.65
Other families	21	30.90

Collection was at one light during the 1969-70 season and totalled 17,226 individuals.

in Lepidoptera we have data from Glenburnie, Ontario, a highly disturbed agricultural area about 20 km from the Perth Road site. These data (Table 1) resemble those of Taylor and Woiwod in so far as there is no indication of an inverse relationship between species abundance and family size such as existed at our Perth Road and Chaffey's Locks sites. In fact at Glenburnie the Noctuidae, the largest family, have the highest mean abundance, but this is largely the result of a single species represented by nearly 6,000 individuals. The apparent direct relationship between family size and species abundance found by Taylor and Woiwod may be the result of small sample size or possibly selective sample choice, for the Rothamsted Survey collections were made at 160 sites⁴, only two of which are reported here⁵. From this same standpoint we should make it clear that we have made collections at only three sites, two in the undisturbed areas described previously³ and the remaining collection at Glenburnie.

The difference between abundance patterns in disturbed and natural habitats is probably a reflection of the increased variance in patch size, characteristic of cultivated areas in which small remnants of the native vegetation persist in the midst of a floristically depauperate agroecosystem. Often the largest patches in such heterogeneous environments

represent novel vegetation resources. For example, in south-eastern Ontario the most important elements of the agricultural grassland are introduced species⁶. Assuming the ability to use such novel resources is fortuitous, then one would expect that the species using these patches would tend to belong to the larger families of the original source fauna and, other factors being equal, these species should be overwhelmingly abundant.

We feel that confirmation of the importance of diffuse competition should only be sought in the analysis of data collected in areas where human disturbance is minimal. We predict that the effects will be most pronounced in homogeneous areas of long term stability, such as tropical rainforest, where variance in patch size is low and thus where one *a priori* expects species equitability to be high.

We thank C. C. Bower for comments.

P. D. N. HEBERT
P. S. WARD
R. HARMSSEN

University of Sydney,
Sydney, NSW 2006, Australia

- ¹ Terborgh, J., *Ecology*, **52**, 23, (1971).
- ² Kempton, R. A., and Taylor, L. R., *J. anim. Ecol.*, **43**, 381 (1974).
- ³ Hebert, P. D. N., Ward, P. S., and Harmsen, R., *Nature*, **252**, 289, (1974).
- ⁴ Taylor, L. R., and French, R. A., *Rept Rothamsted. expl. Stn 1973. Pt 2*, 240 (1974).
- ⁵ Taylor, L. R., and Woiwod, I. P., *Nature*, **257**, 160 (1975).
- ⁶ Beschel, R. E., *Symp. Geobotany of Southern Ontario*, (Royal Ontario Museum, Toronto, 1969).

Gravitational analogue of the magnetic force

SALISBURY and MENZEL¹ have considered the case of a pair of charged masses separated by a distance, r , moving together at a velocity, v , in the direction perpendicular to the line joining their centres. The charges and masses were chosen so that the electrical and gravitational forces cancel one another in the moving coordinate system. Using only special relativity, Salisbury and Menzel arrived at the conclusion that the gravitational equivalent of the magnetic force is

$$\gamma^2 \beta^2 (2 - \beta^2) (G m_1 m_2 / r^2)$$

where m_1 and m_2 are the rest masses, $\beta = v/c$, $\gamma = (1 - \beta^2)^{-1/2}$, and G is the gravitational constant.

The correct special relativistic expression is arrived at as follows. The trans-

formation equations for forces are well known². The gravitational force on one of the masses, in their own reference frame, is Gm_1m_2/r^2 . In the 'laboratory' frame, with respect to which their velocity is v , the force is again along the line joining the centres of the masses and is $Gm_1m_2/(\gamma r^2)$.

An observer in the laboratory frame would find that the masses were γm_1 and γm_2 . If he calculated the gravitational force with the usual formula, he would find it to be $\gamma^2 Gm_1m_2/r^2$, which is larger than the true force by the quantity

$$X = [\gamma^2 - (1/\gamma)]Gm_1m_2/r^2$$

But X is not the gravitational equivalent of the magnetic force. If m_1 carries a charge q_1 and m_2 a charge q_2 , the magnetic force on q_2 is q_2vB_1 , where B_1 is the magnetic induction resulting from q_1 (ref. 3), or

$$\gamma\beta^2q_1q_2/(4\pi\epsilon_0r^2)$$

If q_1 and q_2 are of the same sign, the electric force on q_2 in the moving frame is repulsive, and the magnetic force in the laboratory frame is attractive. Since the gravitational force in the moving frame is attractive, the gravitational analogue of the magnetic force in the laboratory frame is repulsive. It is given by replacing $q_1q_2/4\pi\epsilon_0$ by Gm_1m_2 in the expression already given, or by

$$\gamma\beta^2Gm_1m_2/r^2 \approx \beta^2Gm_1m_2/r^2$$

where m_1 and m_2 are again the rest masses since in the moving frame the masses are fixed with respect to one another.

The quantities γ_1 and γ_2 (see ref. 4) referred to by Salisbury and Menzel come from general relativity. They cannot be arrived at using only special relativity.

PAUL LORRAIN

Département de physique,
Université de Montréal,
Montréal, Canada

¹ Salisbury, W., and Menzel, H., *Nature*, **252**, 664 (1974).

² Lorrain, P., and Corson, D., *Electromagnetic Fields and Waves*, 225 (Freeman, San Francisco, 1970).

³ *ibid.*, 261.

⁴ Blokland, A., *Astrophys. Space Sci.*, **12**, 221 (1971).

MENZEL AND SALISBURY REPLY—Professor Lorrain is correct in his assertion that the gyron force between moving masses is repulsive. We show that in our paper. But, his suggestion that the transformation for magnetic forces between moving charges also applies to moving masses is incorrect. The magnetic force

between charges is proportional to the product of the equivalent currents, q_1v_1 and q_2v_2 . In our simplified presentation, v_1v_2 happens to equal v^2 . A similar identity occurs with the increase in mass caused by the kinetic energy of the motion. That increase in mass produces an extra force between the bodies. The force is proportional to the mass-current product, $m_1m_2v^2$ in our simple case, and must be distinguished from the gyron or magnetic-like repulsion.

That the magnetic force and the gyron force cannot have exactly the same coefficients should be clear from the fact that both experimentally and theoretically, electric charge is conserved under a Lorentz transformation, whereas mass increases with velocity.

The criticism that we are solving a problem in general relativity by means of special relativity is not valid. Particle accelerators demonstrate clearly that high accelerations and great mass increases occur. Thus, general relativity can be approached through special relativity; Einstein used the approach to obtain his famous law: $E = mc^2$.

General relativity theory is clearly incomplete because it predicts that mass is conserved in the same way as electric charge. The new Yilmaz theory of general relativity corrects this error and gives a conservation law of mass-energy and momentum that allows for a smooth transition between special and general relativity¹. Further, the new theory satisfies the experimental criteria.

Any form of relativity must have a firm experimental basis. The invariance of electric charge is well founded experimentally. The change of mass with velocity is known to a high degree of accuracy; it materially affects the design, operation, and observation of a wide variety of particle accelerators.

DONALD H. MENZEL
WINFIELD W. SALISBURY

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

60, Garden Street,
Cambridge, Massachusetts 02138

¹ Yilmaz, H., *Nuovo Cim.*, **7**, 9 (1973).

Embryonic chick tibiae in steady electric fields

WATSON *et al.*¹ report that the growth rate of embryonic chick tibiae *in vitro* is enhanced by a pulsed electric field of 1,000 V cm⁻¹ but not by a static field. We suggest that there is a simple reason why no enhancement should be expected in the static case. This has to do with the ability of the cultures to sustain a field.

Any field produced within the tissue will decay exponentially with a relaxation time constant²

$$\tau = \epsilon_0 k / \sigma$$

$$= 8.8 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$$

where σ is the conductivity of tissue, k the dielectric constant of tissue and ϵ_0 the permittivity of free space.

There seem to be no published data on the conductivities and dielectric constants of uncalcified embryonic bone, but Schwan³ has published data on the electric properties of other tissues (for example, lung, muscle and liver). Representative values at very low frequencies (1–10 Hz) are $\sigma = 10^{-3}$ mho cm⁻¹ and $k = 10^6$. It seems reasonable that values for uncalcified chick tibia should have the same orders of magnitude. Converting σ to m.k.s. we derive a relaxation time constant $\tau \approx 10^{-4}$ s. The corresponding value for a dielectric like fused quartz is in excess of 10^6 s.

This shows why enhancement of growth is not to be expected in a steady field. During the 9-d growth period *in vitro* there is no field within the bone, no charge movement within or on the bone, and so no bioelectric command signal to the bone, except at the instants when the field is switched on or off.

Schwan's values for k may perhaps be treated with caution, but assigning more typical values to the dielectric constant (say $k = 10$) gives essentially the same result—in the steady field there is no transducer mechanism available.

RONALD I. DUNCAN
ALEXANDER D. MACMILLAN

Department of Biophysics,
University of Western Ontario,
London, Ontario, Canada

¹ Watson, J., de Haas, W. G., and Hauser, S. S., *Nature*, **254**, 331–332 (1975).

² Pauofsky, W. K. H., and Phillips, M., in *Classical Electricity and Magnetism*, ch. 7 (Addison-Wesley, Massachusetts, 1962).

³ Schwan, H. P., in *Advances in Biological and Medical Physics*, V (Academic, New York, 1957).

reviews

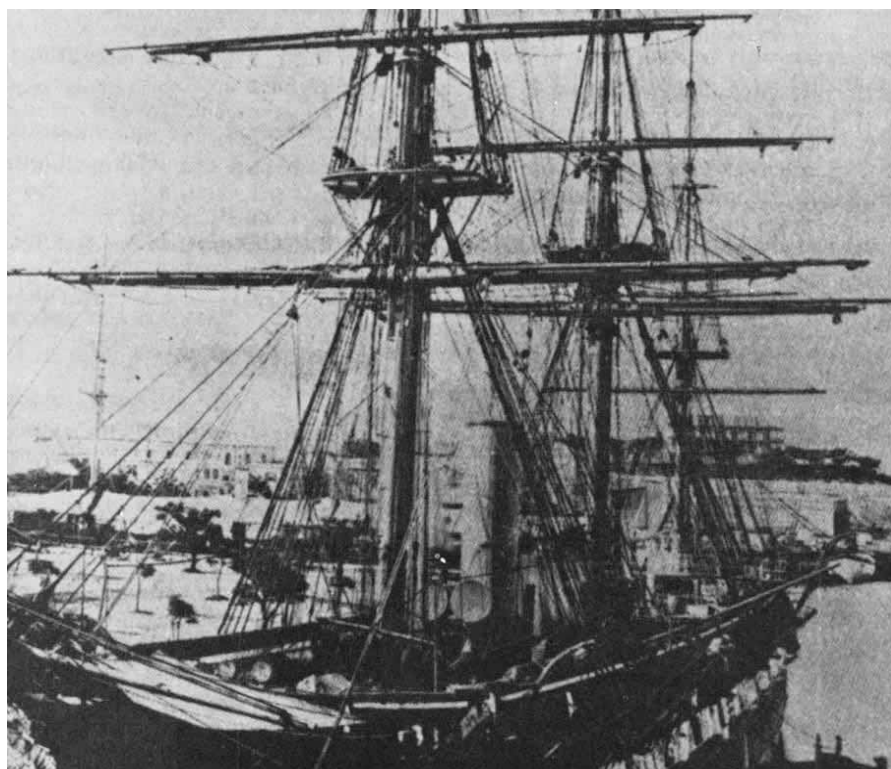
Oceanography: edge of an unfamiliar world

Courtesy Trustees of the British Museum (Natural History)

WE have become used to publishers coyly hiding the dates of publication of their books on the back of the title page; they believe, I suppose, that no one buys books when they are more than a year old. The publishers of this book* have extended the idea and concealed the fact that it is a reprint of one first published in America, by another company in 1973 (though they do say "© 1973"). This practice is dubious in relation to the purchaser; for the author, in this instance, the practice is grossly unfair. The reader looks for references to Margaret Deacon's book, which has become the standard work on the history of oceanography, and does not find them. The reason is that copies of her book only reached the US at the time that this book went to press so the author is, quite unjustly, made to seem ignorant of things one would expect to find in a book published in 1975.

The book covers the period from about 1830 to the 1970s and does not touch on the work of the 17th and 18th centuries. That is fortunate as the earlier period has been fully covered by Margaret Deacon. The opening chapters, on the early years of what eventually became the Coast and Geodetic Survey and the Hydrographic Office of the Navy, are, perhaps, the most instructive and original part of the book. There, Ms Schlee has gone back to the primary sources and one can see the back history of oddities of organisation and of disputes which lasted almost to the present time. The account of the "infamous United States Exploring Expedition" and of the shortcomings of M. F. Maury as a theorist are particularly interesting.

In the later parts of the book she demonstrates an irritating tendency to quote quite dubious and often inaccessible secondary sources when better information is easily available. For example, for an interesting quotation from



RV Challenger, one of the earliest oceanography research vessels, at Bermuda in 1873

Darwin the reader is referred to *The Darwin Reader* which contains, presumably, extracts from Darwin's works. Again, an improbable quotation from Halley is taken from *The Last Cruise of the Carnegie* where it occurs, but without any reference. Occasionally, the author is led into error by such sources: for example, Edmond Halley, with his name spelled Edmund (a form he never used in English) sails to the Antarctic in the *Paramour Pink* (a Pink is a kind of ship, not the name of a ship); both are venerable errors. The source of other errors is less clear, as when Ritter is said to have founded Scripps and to have been an "instructor in biology at the University of California at San Diego" (he was a professor at Berkeley, and UCSD was founded 60 years later by a professor from Scripps). This error is part of an eastern US and western European bias; for Ms Schlee everything west of the Ohio is Indian Country. These small errors are not of much importance but they do produce a feeling of insecurity in the reader and it is only fair to say that my doubts on several other

matters proved unfounded (for example, I doubted that St Vincent was First Lord in 1804).

The author has, perhaps, not firmly decided for whom the book is written: much of it is serious and reliable history, but in places she feels the story is getting dull and lapses into a more popular style with a wealth of irrelevant detail derived, remotely, from Hemingway and his imitators. For example: "A light snow was falling on Boston Harbour as two tugboats cast off their heavy docking lines . . ."; I am sure that she knows that it was snowing and that the lines were heavy—but that is the language of journalism rather than of history.

Perhaps I have overstressed the things that, to me, seem shortcomings; it is an interesting and readable book, contains many things that will be new to most readers and should be read by anyone with a serious interest in oceanography and the history of its development.

Some of the figures are little more than black smudges; they were only slightly better in the American edition.

E. Bullard

**A History of Oceanography: The Edge of an Unfamiliar World*. By Susan Schlee. Pp. 398. (Robert Hale: London, February 1975.) £5.

Numbers of people

Population Policy in Developed Countries. Edited by Bernard Berelson. Pp. xiii+793. (A Population Council Book.) (McGraw Hill: New York and London, 1974.) \$17.50.

THIS is a collection of essays on the present demographic situation and the policies relevant to population growth, composition and distribution in 24 'advanced' countries (including Argentina, Greece and Spain). Bernard Berelson contributes an introduction and a very sensible and comprehensive summary.

It is clear from this collection that Britain, the Netherlands and the United States are the only countries in which there has been any marked concern during the 1960s with overpopulation, and even in those countries, the concern is muted and becoming more so. In all others, the concern is more with increasing the total population or, as in the cases of Israel and South Africa, with redressing a perceived imbalance between different groups. The reasons for this are various. They include an anxiety about future manpower (even the Netherlands, although worried by high density, encourages immigration), an anxiety about political status (most evident in Argentina and Israel, but by no means absent from many other countries), and a more diffuse anxiety about the dangers of some sort of inertia from stationary or declining numbers. It is interesting that the arguments reviewed for and against rising numbers are all, without exception, inconclusive. The sanest stance is perhaps taken by the Swedes, who seem to be entirely agnostic about the reputed effects of different demographics and concern themselves instead with the maintenance and improvement of the quality of life for those who are already living.

It is also clear from these essays, however, that any country wishing to do anything about its rate of population growth is faced with considerable difficulties. Punitive policies (such as that recently introduced in Singapore) are out of the question for moral and political reasons. And less punitive policies seem to have uncertain or weak effects. There is little moral or political room for manoeuvre over mortality; providing adequate facilities for birth control is no more likely to reduce fertility than is withdrawing them likely to raise it; and although there is considerable political, if not moral, room to alter migration flows, emigration cannot be controlled easily. That leaves immigration, which many societies have used to alleviate their problems. It seems likely, however, that in all but a few cases (one of which, interestingly, seems to be Finland) the supply of

potential immigrants is drying up, and where it is not, the potential immigrants are highly undesirable.

This volume therefore gives the impression that national concerns about population are founded upon very uncertain arguments, and that even where action is considered desirable, it is rarely possible to a decisive extent. The interest, perhaps, for scientists is that so few countries pay any attention to the environmental consequences of demographic patterns. In my opinion, that attitude is correct: the connection between population and the environment is at best an indirect one; accordingly, direct measures to protect or improve the environment need be little influenced by population.

G. P. Hawthorn

Man and fire

Fire and Ecosystems. (Physiological Ecology: A Series of Monographs, Texts and Treatises.) Edited by T. T. Kozlowski and C. E. Ahlgren. Pp. xii+542. (Academic: New York and London, December 1974.) \$39.50; £18.95.

To the wildlife conservationist, fire is at the same time a valuable tool and a feared enemy, and it is this love-hate relationship which man has towards fire which forms the underlying theme of this book. It is a collection of regional essays by many eminent ecologists, most of whom are directly concerned with the practical side of forestry, range management or agriculture, and it provides a very extensive review of a subject, which demands the attention of all who are interested in the relationship between man and his environment.

Following a brief introduction there are three chapters, by Viro, Ahlgren and Bendell, of a general nature, which concern the influence of fire on soil, soil organisms, and birds and mammals, respectively. Of these the first, on soil, is very much more limited in its coverage than one might have hoped. Almost all the data quoted concerns podzols in boreal forest areas of Fennoscandia (90% of the literature quoted in the bibliography for this chapter is Scandinavian or Finnish). Although much has been contributed to our knowledge of this subject by scientists from that part of the globe, this presentation represents a very narrow approach to what purports to be a general survey of a very important aspect of the ecology of fire. The chapters on soil microbes and, particularly, on birds and mammals are, however, very full and serve as useful reviews.

There follow, in the main body of the book, sections dealing with the influence of fire on the major biomes of the world, grassland, deciduous and coniferous forests, chaparral and Mediterranean vegetation, and savanna and desert.

Apart from the chapter by Vogl on grasslands, these sections each tend to be devoted to specific geographical regions: for example, the section on forests is divided into four chapters, each dealing with one of the major forested areas of the United States. Although this system of subdivision has undoubtedly made it easier for each of the authors to concentrate on one area of the extensive literature and has also facilitated the task of editing, I feel that some potential has been lost as a result. It would have been useful if some chapters dealing with more general themes could have been incorporated, after the fashion of the first four chapters. But perhaps this attitude is inevitable in west European ecologists as their region is so markedly neglected. Given such a geographically subdivided approach, however, it is sad that space was not found for a chapter dealing with the influence of fire upon heathland and moorland ecology, a subject concerning which there is a wealth of literature. In fact, it is touched upon only briefly, for example, in Bendell's chapter where two pages are given over to the red grouse.

The final chapter, by Kayll, on the use of fire in land management, attempts the kind of synthesis which could have been profitably applied to various other topics in the book. This chapter deserved more space.

Overall, one is left with a book which covers an enormous literature and which is rich in specific examples of fire-adapted species and ecosystems from several parts of the world, but mainly from North America. North American conservationists seeking such specific information will undoubtedly welcome the arrangement of text. Those who seek more general information, however, perhaps relating to fire temperatures and influences upon succession or nutrient cycling processes, will have to collate the numerous, brief references scattered through the text. There are, however, two pervading features for which credit is presumably due to the editors: first, the emphasis upon historic and pre-historic use of fire by man in various biomes and, second, the present day value of fire as a tool in habitat management. These themes are easily traced through most of the chapters, the former reaching a splendid climax in Naveh's biblical and classical account of the history of the use of fire in the Mediterranean region.

The escalating price of this series of monographs on physiological ecology must be remarked upon. This is particularly unfortunate as it is likely to remove them from a rich potential market among nonspecialists, ecologists and conservationists, in whose hands several volumes in the series would have proved particularly valuable.

Peter D. Moore

Oceanology, meteorology and climatology

Oceanology, vol. 1. (Geophysics Series.) Edited by A. P. Kapitsa and P. S. Lineykin. Pp. 127. (Hall: Boston, Massachusetts, January 1975.) \$19.00.

Meteorology and Climatology, vol. 1. (Geophysics Series.) Edited by I. P. Danilina and A. P. Kapitsa. Pp. i+224. (Hall: Boston, Massachusetts, January 1975.) \$29.00.

THESE two books are the first volumes of a proposed series of reviews of recent scientific achievement in geophysical subjects: oceanology, meteorology and climatology, physics of the earth, glaciology, and geomagnetism and the upper atmosphere. Published by the Russian All-Union Institute for Scientific and Technical Information (VINITI) they continue the Summaries of Scientific Progress which first appeared in 1964. Chapters are contributed by Russian authorities in the relevant fields and outline briefly, with some comment, advances made in the two or three years before the original publication in Russian; the volumes thus refer to literature published in the late 1960s and early 1970s. Although the emphasis is placed on Russian work, there is also discussion of research completed in other countries.

The volume entitled *Meteorology and Climatology* includes chapters on physical, satellite and agricultural meteorology, climate and climatic change, photochemistry and the ozone-sphere, and glaciers. The *Oceanology* volume covers sea-atmosphere interaction, sea ice, sea straits, sea forecasts and oceanographic technology. This choice of topics seems somewhat arbitrary, but will probably vary in future volumes. The presentation ranges between the extremes of complete specification of the then current state of knowledge in the particular field and terse chronological summaries of recently published literature, with little reference to earlier research.

Although these works provide insight into the development of various aspects of the sciences, the publication in English some years after their first appearance in Russian rather dates the material discussed. It is felt that researchers in these fields will already be familiar with literature published, even in Russian, five years ago and that their primary interest will be historical.

But value of future volumes in the series would be greatly enhanced if a shorter delay between Russian and English publication could be achieved.

P. M. Kelly

Iron in organisms

Iron in Biochemistry and Medicine. Edited by A. Jacobs and M. Worwood. Pp. xiv+769. (Academic: London and New York, October 1974.) £15.20; \$39.25.

IRON is one of the trace elements present in mammals. It is a transition metal and as such it exists in several oxidation states and forms many metallo-protein complexes. The fascinating multiplicity of structure and function in iron compounds has been investigated by many research workers in widely separated disciplines and their efforts to elucidate various aspects of iron metabolism have yielded a vast amount of valuable information. It has become difficult to keep track of all the published data in several rapidly expanding areas of iron metabolism, but the excellent collection of reviews in *Iron in Biochemistry and Medicine* now makes this task much easier.

In this book 35 active investigators have reviewed the present state of knowledge in their particular areas of interest for the benefit of their colleagues in the same field and for those working in other disciplines. The book comprises twenty chapters, each containing a review of one topic. The topics selected all concern major aspects of iron metabolism.

The biochemistry of iron compounds is discussed in the first half of the book: the structure and function of inorganic iron, transferrin, ferritin, haemosiderin, haem, haem-proteins and non-haem iron proteins are all reviewed in great detail. The chapter on the relationship between iron and other trace metals completes the biochemical section. This is followed by reviews on the physiology and pathology of iron metabolism: iron absorption, iron deficiency and its manifestations and effects, its epidemiology and treatment, iron metabolism in infancy and childhood, the iron and the reticuloendothelial system, and iron overload. Finally, in the last three chapters, the kinetics of iron metabolism, the relationship between iron and infection, and genetic abnormalities of iron metabolism in mice are reviewed.

The book is uniformly well written, with surprisingly little repetition, a tribute to the editorial policy. Many tables and a modest number of illustrations, all thoughtfully prepared, are of great help to the reader, and the references at the end of each chapter are extensive though not comprehensive.

It is perhaps the aggregation of data from widely separated subjects which makes this book so valuable. I would certainly like to have it to hand if I needed to quote the data on iron metabolism from outside the area of my personal interest.

I strongly recommend this book as useful reading for scientists working in

this field. The clinician will find a wealth of biochemical information on the iron proteins he is trying to study by simple means in his patients. The biochemist will learn a lot about the significance of abnormalities of iron metabolism found in diseases. And I am sure that both will obtain considerable help in planning and executing future research.

B. Brozović

Hadron physics

High Energy Hadron Physics. By Martin L. Perl. Pp. xviii+562. (Wiley-Interscience: New York and London, December 1974.) £12.00.

THE nature of the strong nuclear (hadronic) interaction has been the subject of extensive theoretical and experimental investigation over the past 20 years and more, and from time to time books have appeared on aspects of this field, usually impressing upon the reader the need for further understanding of the theoretical complexities involved. This monograph by Professor Perl, an international authority in the field, helps considerably in that respect, and will be of interest and value to postgraduate students and workers at the postdoctoral level. The completion of this book, a *tour de force*, clearly required considerable stamina besides intricate academic knowledge. The author moves along with obvious energy and enthusiasm from the outset to the end, some 500 pages later, generating *en route* considerable respect for both Perl himself and his subject. Were anyone to doubt the usefulness and value of sabbatical leave, let him obtain a copy and read this book. Without such support, most practising experimental physicists would simply not have the time to create work of this quality and detail.

The standard of the book is such that the reader needs a knowledge of the basic theoretical and mathematical background material in elementary particle physics, such as one may acquire from lectures at the first year postgraduate level. Although the style of writing is often conversational, the book demands serious concentration; the considerable formal mathematical manipulation in some chapters makes the going heavy and will demand the continued perseverance of the student. I feel that the low energy nuclear or astrophysicist, who picks up this book and sees from the fly leaf that it will be an "invaluable reference" to him, could well be in deep water very soon.

The book, which contains a reasonably balanced mixture of experimental data and theory, may seem expensive but at present standards it is not excessively so. I hope, and expect, to find the book retained by science libraries for reference.

W. Galbraith

Topics in bioinorganic chemistry

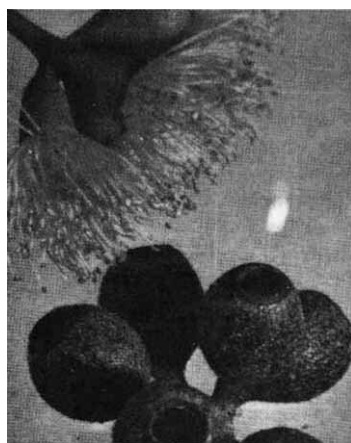
Techniques and Topics in Bioinorganic Chemistry. (Aspects of Inorganic Chemistry.) Edited by C. A. McAuliffe. Pp. xv+351. (Macmillan: London and Basingstoke; February 1975.) £20.00.

THE frontier between inorganic chemistry and biochemistry has been crossed so often that the border is now ill-defined. It is too early to know how the buffer state, which bioinorganic chemistry or inorganic biochemistry may be considered to constitute, will evolve. Certainly, the subject is fashionable. But that which is fashionable is not necessarily respectable, the latter description, evocative of safe, solid virtues, being more often applied to the mainstreams of the parent subjects. But irrespective of prejudices it is impossible to ignore the importance of those elements which normally constitute the province of inorganic chemists, to biological processes. The roles of proteins containing iron and copper in all manners of redox reactions and oxygen transport are well known, and the extent to which other ions, such as zinc, are involved is slowly being revealed. Many other elements, for example, selenium, are known or suspected to be required but their functions ill-defined.

This book is one of several which have appeared recently and which seek to attract attention to the current excitement for the subject. In the first chapter M. W. Makinen attempts ambitiously to relate the functions of some proteins to structural details derived from X-ray diffraction studies occasionally reinforced by information gleaned from spectroscopic investigations. J. M. Pratt, in a chapter entitled "Principles of Catalysis by Metalloenzymes", considers the haemoglobins and myoglobins, catalase and peroxidase, and nitrogenase. The biochemical function of molybdenum is described by F. L. Bowden in a well planned review. Since the environments of molybdenum in proteins such as xanthine oxidase and nitrogenase are unknown, speculation about the role of molybdenum in these proteins is subdued. Some interesting chemistry of molybdenum is, however, included; the relevance to the biochemical function is not overstated. J. Webb discusses a number of complexes and proteins involved in iron transport and storage, with considerable emphasis on the electronic structures of the interacting iron ions. The volume is completed by an all-too-brief introduction by S. J. Ferguson to the use of nuclear magnetic resonance

spectroscopy in the investigation of the structures of proteins in solution.

Books containing chapters by different authors often suffer from two defects. First, the delay between completion of the manuscripts and publication leads, in a rapidly developing subject, to redundant speculation. Second, although the editor in his introduction seeks to justify overlap between the contents of the various chapters, this may prove irritating to those prepared to read through the book. For example, the binding of molecular oxygen to iron in myoglobin and haemoglobin is considered at length in chapters 1 and 2, as is nitrogen fixation in chapters 2 and 3. But as it is likely that the volume will be used as a source of review articles, this criticism may not be pertinent. **H. A. O. Hill**



Eucalyptus jucunda C. A. Gardner, usually 2–3.5 m, but sometimes up to 8 m high. Photo shows stamens and elliptical fruiting bodies (1.5 × 1.2 cm). Taken from *Eucalypts of the Western Australia Goldfields* by G. M. Chippenale. Pp. iv+216. (Australian Government Publishing Service: Canberra, 1973.) A\$3.75.

Casting light on structures

Biochemical Spectroscopy. By Richard Alan Morton. Vol. 1: pp. xvi+1–381; vol. 2: pp. 382–873. (Adam Hilger: London, March 1975.) £80.00 for set.

THE title *Biochemical Spectroscopy* may today conjure up an image of a work dealing fairly exhaustively with the practical and theoretical aspects of the spectroscopy, primarily, of current molecular biology. It might, for instance, emphasise the applications in that field of the more modern techniques of ESR, Mössbauer and NMR, and perhaps give as much weight to

circular dichroism, optical rotatory dispersion and Raman as to the older basic techniques.

This book is, however, of a quite different kind. Essentially, it consists of a large number of monographs, grouped rather arbitrarily into longer sections. Of these, some, like those entitled 'Water and Sunlight in a Biological Context' and 'Electronic Absorption Spectra', give background material of a relatively generalised kind but most of them are groups of monographs, many quite short, on classes of natural products and the light that spectroscopic methods have cast (and in a few cases might yet cast) on their structures, functions and interrelations. The range of topics is wide, and it is revealing to see in how many and diverse fields the author has himself made contributions in a laboratory career lasting 50 years. A chemist with no special interest in spectroscopy could do worse than to turn first to this book if he should have to deal with a natural product belonging to a known but unfamiliar class; the treatment of the chemistry is always interesting, if necessarily incomplete, and the references are excellent. The writing is clear and straightforward, and, though the bias is towards classical visible and ultraviolet spectroscopy, developments based on other techniques are not neglected.

In places the reader encounters unexpected hurdles, however. Some of these arise from a failure to number, and thus to correlate with the text, the numerous structural formulae embedded in it, and in places the lack of either names or reference numbers, coupled with a sprinkling of errors, makes the going hard indeed for a reader unfamiliar with the field. The sources of much of the tabulated data are not given, and some sections wear the antique look which is conveyed by the bulgy contours of 30 or 40 year-old spectral diagrams; these, however, normally occur where the more modern data which might otherwise supersede them are incomplete. Although in places some updating of material is surely needed, a prime defect is the plethora of small errors and misprints which defaces this handsomely produced—and extremely expensive—pair of volumes.

The price is such, in fact, that the volumes can only be considered as a library reference work. This is a great pity, since a deal of pleasure and information is to be had from random browsing. It is, however, clearly impossible to suggest cuts and economies in presentation which could result in a price reduction by the order of magnitude which is needed to make the volumes as accessible as they deserve to be. **E. A. Johnson**

announcements

Award

The **Kelvin Gold Medal** has been awarded to **C. S. Draper** for his work on the problems of guidance and control in space.

Appointments

R. C. Newman has been appointed Professor of Physics at Reading University.

D. Givol has been named the first incumbent of the Altschuler Chair of Immunochemistry.

J. D. Gillett has been appointed the first Pro Vice-Chancellor of Brunel University.

Miscellaneous

Culture Collections. The United Kingdom Federation for Culture Collections, designed to assist microbiologists, is now in existence. Further information can be obtained from Dr I. J. Bonsfield, Torry Research Station, PO Box 31, 135 Abbey Road, Aberdeen, Scotland.

International meetings

September 29–October 1, **Biological implications of metals in the environment**, Richland, Washington (Miss J. H. Rising, Biology Department, 331 Building, Battelle-Northwest, Richland, WA99352).

October 7–9, **Techniques for the microbiological examination of foods, pharmaceutical and cosmetic products**, London (Scientific Symposia Ltd., 121 King Street, London W6 9JG, UK).

October 20–24, **Clean air and pollution control**, Brighton (The Secretary, National Society for Clean Air, 136 North Street, Brighton BN1 1RG, UK).

October 29–November 1, **Chemical evolution of the early precambrian**, Maryland, US (Dr Cyril Ponnamperna, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, College Park, Maryland 20742).

October 25–29, **Medical radionuclide imaging**, Los Angeles (The Secretary, International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

October 27–31, **Heavy metals in the environment**, Toronto (Mr K. Ward, Executive Secretary, National Research Council of Canada, Ottawa, Canada K1A 0R6).

Person to Person

Printing of mathematics. Could anyone loan or sell me a copy of *The Printing of Mathematics* (Oxford, out of print)? Also any other books on mathematical printing. Will pay postage. Vic Cresswell, Dawkins Typesetters, Graphic House, Southwark Street, London SE1 or (01)-407 7525.

Seeds and latex. Investigator will appreciate knowing where to obtain seeds and latex (1–2 kg of each) of *Hura crepitans* (*Hura brasiliensis*) (Professor F. Stirpe, Istituto di Patologia generale, Via S. Giacomo 14, 40126 Bologna, Italy).

Endoplasmic reticulum. Biologists writing a book on the structure of living cells would be grateful to know of any publications which have indicated that the endoplasmic reticulum might be an artefact. (Dr H. Hillman and Mr P. Sartory, Unity Laboratory, Department of Human Biology, University of Surrey, Guildford, Surrey GU2 5XH).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 30, **Recent chemistry of natural products, including tobacco**, Richmond (Nicholas J. Fina, The Phillip Morris Research Center, PO Box 26853, Richmond, Virginia 23261).

October 30–31, **Comparative biology of skin**, London (Dr R. I. C. Spearman, The Zoological Society, Regent's Park, London NW1 4RY, UK).

Reports and publications

Great Britain

Timber Research and Development Association. Annual Report for 1974. Pp. x+16. (High Wycombe, Bucks: Timber Research and Development Association, 1975.) [27]

1975 Conference on Dielectric Materials, Measurements and Applications, 21–25 July 1975, Churchill College, Cambridge. (Organized by the Science, Education and Management Division of the Institution of Electrical Engineers, in association with the Institute of Electrical and Electronics Engineers (United Kingdom and Republic of Ireland Section); the Institute of Physics; and the Institution of Electronic and Radio Engineers.) Pp. xii+336. (London: The Institution of Electrical Engineers, 1975.) [27]

Explore the New Forest. Edited by Donn Small. (An official guide by the Forestry Commission.) Pp. 125. (London: HMSO, 1975.) £1.85 net. [37]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1286: The Influence of Pore-Water Tension on the Strength of Clay. By A. W. Bishop, N. K. Kumapley and A. El-Ruwayih. Pp. 511–554+Plate 17. UK £3.95; Overseas £5.10. Vol. 278, No. 1287: The Initial-Value Problem for the Korteweg-de Vries Equation. By J. L. Bona and R. Smith. Pp. 555–604. UK £1.95; Overseas £2. (London: The Royal Society, 1975.) [37]

The Lister Institute of Preventive Medicine. Report of the Governing Body, 1975. Pp. 22. (London: The Lister Institute of Preventive Medicine, Chelsea Bridge Road, SW1, 1975.) [47]

Association of the British Pharmaceutical Industry. Annual Report, 1974/75. Pp. 44. (London: Association of the British Pharmaceutical Industry, 162 Regent Street, 1975.) [77]

Potato Marketing Board. Machinery Directory. Pp. 44. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) 15p. [87]

Rowett Research Institute. Annual Report of Studies in Animal Nutrition and Allied Sciences, Vol. 30, 1974. Pp. 143. (Bucksburn, Aberdeen: Rowett Research Institute, 1975.) £1; \$2.30. [87]

National Vegetable Research Station. 25th Annual Report 1974. Pp. 166. (Wellesbourne, Warwick: The British Society for the Promotion of Vegetable Research, National Vegetable Research Station, 1975.) £1.25. [107]

Bulletin of the British Museum (Natural History). Geology, Vol. 26, No. 2: The Shell Structure of the Liassic Ammonite Family Dactyloceratidae. By M. K. Howarth. Pp. 45–67+10 plates. (London: British Museum (Natural History), 1975.) £2.70. [107]

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 10: Luminescence from Chromium Doped Yttrium Aluminium Garnet. By M. O. Henry, J. P. Larkin and G. F. Imbisch. Pp. 97–106. 35p. Vol. 75, Section B, No. 15: The Distribution and Morphometric of Spittle Bugs on Irish Blanket Bog. By N. Nixon, Elaine F. Okely and Ruth M. Blackith. Pp. 305–315. 29p. (Dublin: Royal Irish Academy, 1975.) [107]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 271, No. 911: A Discussion on Forests and Forestry in Britain. Organized by G. D. Holmes, P. F. Wareing and J. L. Harley. Pp. 45–232. (London: The Royal Society, 1975.) UK £7.75; Overseas £7.95. [107]

European Spectroscopy News, Vol. 1, No. 1, 1975. Pp. 1–32. Edited by P. M. Williams. Published Bimonthly from July 1975. (London: Heyden and Son, Ltd., Spectrum House, Alderton Crescent, NW4, 1975.) Free to all Practising Spectroscopists. [107]

London and Home Counties Regional Advisory Council for Technological Education. Science Education in the Region, 1975/76. Pp. 55. (London: London and Home Counties Regional Advisory Council for Technological Education, 1975.) 70p. [147]

Responsibilities of Industry in the Field of Health. By Dr J. H. Briggs and Dr. Robert Murray. (Foundation Dialogues.) Pp. 16. (London: Foundation for Business Responsibilities, Portland House, Stag Place, SW1, 1975.) 25p. [157]

Union Internationale des Laboratoires Independants. Register of Members 1975. Pp. 237. (London: Union Internationale des Laboratoires Independants, Ashbourne House, Alberon Gardens, NW11, 1975.) [157]

Freedom and Restraint in Broadcasting: The British Experience. By Sir Michael Swann, FRS. (The 'Queen' Lecture given in the Kongresshalle, Berlin, 29 May 1975.) Pp. 31. (London: BBC, 1975.) [157]

Public Health Laboratory Service. Monograph Series No. 8: Isolation of Salmonellas. By R. W. S. Harvey and T. H. Price. Pp. 52. (London: HMSO, 1974.) £1.50 net. [157]

Central Water Planning Unit. First Annual Report for the year ending 31st March 1975. Pp. 34. (Reading: Central Water Planning Unit, Reading Bridge House, 1975.) [167]

- The Family Planning Association 43rd Report and Accounts, 1974/1975. Pp. 28. (London: The Family Planning Association, 27/35 Mortimer Street, W1, 1975.) [167]
- The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. The Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom in 1976-77. Pp. 304. (London: The Association of Commonwealth Universities, 1975. Published for the Committee of Vice-Chancellors and Principals. Available from Lund Humphries, The Country Press, Drummond Road, Bradford BD8 8DH.) £2.90. [167]
- International Tin Research Council. Annual Report for 1974. Pp. 52. (Greenford, Middx.: Tin Research Institute, 1975.) [177]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 271, No. 912: A Discussion on the Pericellular Environment and Its Regulation in Vertebrate Tissues. Organized by Dame Honor Fell, FRS, and J. T. Dingle. Pp. 233-410 + plates 1-24. (London: The Royal Society, 1975.) [177]
- Flora of Tropical East Africa. Edited by R. M. Polhill. Oliniaceae. By Dr. B. Verdoorn. Pp. 4, 15p. Vahliaaceae. By D. M. Bridson. Pp. 6, 15p. (London: Crown Agents for Oversea Governments and Administrations, 1975. Published on behalf of the East African Community.) [187]
- British Waterways Board. Energy Conservation—The Inland Waterway Role. Pp. 15. (London: British Waterways Board, Melbury Terrace, NW1, 1975.) [187]
- The NRPB Automated Thermoluminescent Dosimeter and Dose Record Keeping System. By J. A. Dennis, T. O. Marshall and K. B. Shaw. Pp. 14. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1974.) [217]
- Aspects of Sewage Treatment. Edited by John Pickford. (Proceedings of the Eighth Public Health Engineering Conference, held in the Department of Civil Engineering, Loughborough University of Technology, January 1975.) Pp. 104. (Loughborough, Leicestershire: Mrs. Rowena Steele, University of Technology, 1975.) £3. [217]
- Public Health Laboratory Service. Monograph Series No. 7: Preservation of Bacteria with Notes on other Micro-organisms. By S. P. Lape and K. F. Redway. Pp. x+121. (London: HMSO, 1974.) £1.50 net. [227]
- Department of Industry. Research and Development Requirements Boards Reports 1974-75. Pp. 36. (London: Department of Industry, Technology Library, Abell House, John Islip Street, SW1, 1975.) [227]
- Communications of the Dublin Institute for Advanced Studies, Series C. Dunsink Observatory Publications, Vol. 1, No. 7: Identification of Variable Stars in the Magellanic Clouds. By C. J. Butler and P. A. Wayman. Pp. 193-206. (Dublin: Dublin Institute for Advanced Studies, 1974.) [247]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 279, No. 1288: A Discussion on the Geology of the English Channel. Organized by Sir Kingsley Dunham and A. J. Smith for the Royal Society and the Marine Studies Group of the Geological Society. Pp. 1-295 + plates 1-19. (London: The Royal Society, 1975.) UK £14.65; Overseas £15.15. [257]
- Science Research Council. Electrochemistry and Electrochemical Engineering—Report of the Second Working Party on Electrochemistry. Pp. 24. (London: SRC Chemical Engineering and Technology Committee, State House, High Holborn, 1975.) gratis [257]
- University of Oxford. Annual Report of the Curators of the Bodleian Library for 1973/1974. Pp. 61. (Oxford: The University, 1975.) £1.50. [287]
- Medical Research Council Annual Report, April 1974-March 1975. Pp. ix+205+12 plates. (London: HMSO, 1975.) £2.75 net. [287]
- Leaching and Reduction in Hydrometallurgy. Edited by A. R. Burkin. Pp. viii+109. (London: The Institution of Mining and Metallurgy, 1975.) £10; \$25. [287]
- British Nuclear Fuels Limited. Fourth Annual Report and Accounts, 1974/1975. Pp. 31. (Risley, Warrington: British Nuclear Fuels Limited, 1975.) [307]
- Bulletin of the British Museum (Natural History). Geology, Vol. 26, No. 3: Neogene Fossil Fishes from the Lake Albert-Lake Edward Rift (Zaire). By P. H. Greenwood and G. J. Howes. Pp. 69-127. (London: British Museum (Natural History), 1975.) £3.35. [307]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 279, No. 1289: A Discussion on Astronomy in the Ultraviolet. Arranged by the British National Committee on Space Research, under the leadership of Sir Harrie Massey and R. Wilson. Pp. 297-485 + plates 11-18. (London: The Royal Society, 1975.) UK £8; Overseas £8.20. [317]

Other Countries

- World Health Organization. Health Project Management: a Manual of Procedures for Formulating and Implementing Health Projects. By J. Bainbridge and S. Sapirie. Pp. 280. (Geneva: WHO, 1974.) [246]
- New Zealand: Department of Scientific and Industrial Research. Bulletin No. 213. Microzonation for Earthquake Effects in Wellington, N.Z. By T. L. Grant-Taylor, R. D. Adams, T. Hatherton, J. D. G. Milne, R. D. Northey and W. R. Stephenson. Pp. 62. (Wellington, NZ: Department of Scientific and Industrial Research, Seismological Observatory, Geophysics Division, 1974.) [246]

- Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 146, No. 10: The Taphonomy and Paleocology of Plio-Pleistocene Vertebrate Assemblages East of Lake Rudolf, Kenya. By Anna K. Behrensmeyer. Pp. 473-578. Vol. 147, No. 1: The Carpoletidae—Early Tertiary Primates from North America. By Kenneth D. Rose. Pp. 1-74. Vol. 147, No. 2: Systematics and Distribution of Ceratioid Angelfishes of the Genus *Chaenophryne* (Family Oneirodidae). By Theodore W. Pietsch. Pp. 75-100. Vol. 147, No. 3: The American Orb-weaver Genera *Larinia*, *Cercidia* and *Mangora* from Mexico (Araneae, Araneidae). By Herbert W. Levi. Pp. 101-135. Vol. 147, No. 4: The Prothorax of Coleoptera—(Except Bostrichiformia—Cucujiformia). By T. F. Hlavac. Pp. 137-183. (Cambridge, Mass.: Harvard University, 1975.) [256]
- Disarmament of Destruction? Armaments and Disarmament. Pp. 21. (Stockholm: Stockholm International Peace Research Institute, 1975.) [256]
- World Health Organization. A Manual on Drug Dependence. (Compiled on the basis of reports of WHO expert groups and other WHO publications.) Edited by J. F. Kramer and D. C. Cameron. Pp. 107. (Geneva: WHO; London: HMSO, 1975.) Sw.fr.24. [266]
- Le Conseil des Recherches et Services Agricoles du Québec. Recherches Agronomiques—Sommaire des Résultats, 1973/1974 (No 19). Pp. 117. (Québec: Ministère de l'Agriculture, 1975.) [266]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 75-1B: Report of Activities, Par vit B. + 333. \$6. Paper 75-26: Uranium Exploration '75 By A. G. Darnley, V. Ruzicka, W. Dyck, E. M. Cameron, K. A. Richardson and R. M. Williams. Pp. vi + 71. \$4.20. (Ottawa: Information Canada, 1975.) [276]
- Man in Southern Africa: The Stone Age. Pp. 24. (Cape Town: South African Museum, 1975.) [276]
- Smithsonian Contributions to Anthropology, No. 17: The Long Sword and Scabbard Slide in Asia. By William Trousdale. Pp. x + 332 (24 plates). (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) \$7.55. [276]
- Smithsonian Contributions to Paleobiology, No. 22: Ultrastructural Studies on Graptolites, 2: The Periderm and Its Derivatives in the Graptoloidea. By Adam Urbanek and Kenneth M. Towse. Pp. iii + 23 + 24 plates. (Washington, D.C. Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) \$1.30. [306]
- World Health Organization Technical Report Series No. 563: Guidelines for Evaluation of Drugs for Use in Man—Report of a WHO Scientific Group. Pp. 59. (Geneva: WHO; London: HMSO, 1975.) Sw.fr.7. [306]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 75-6: The Geosciences in Canada—1974. (A Status Report prepared by the Canadian Geoscience Council.) Edited by E. R. W. Neale, A. C. Clague and H. R. Wynne-Edwards. Pp. 51. (Ottawa: Information Canada, 1975.) \$2.40. [306]
- Berichte des Deutschen Wetterdienstes. Nr. 136 (Band 17): Aeroklimakarten der Druckflächen bis 10, mar und Vertikalprofile für den Raum Nordatlantik-Europa-Naher Osten, Mittlere Windvektoren, Mittel und Streuungswerte von Geopotentieller Höhe und Temperatur. Von Hans Guss. Pp. 13 mit 4 tabellen im Text, 19 Abbildungen und 72 Karten im Anhang. (Offenbach (Main): Selbstverlag des Deutschen Wetterdienstes, 1975.) [306]
- John F. Kennedy Institute: Center for International Studies. A Program for Research, Education and Dialogue—Seventh Annual Report. Pp. 65. (Tilburg, The Netherlands: John F. Kennedy Institute, 1975.) [17]
- World Health Organization. Methods Used in the USSR for Establishing Biologically Safe Levels of Toxic Substances. (Papers presented at a WHO Meeting held in Moscow from 12 to 19 December 1972.) Pp. 171. (Geneva: WHO; London: HMSO, 1975.) Sw.fr.30. [17]
- United Nations Environment Programme. Report of the Governing Council of the United Nations Environment Programme on the Work of Its Third Session, Nairobi, 17 April to 2 May 1975. Pp. 131. (Nairobi: UN Environment Programme, 1975.) [17]
- Records of the Australian Museum. Vol. 29, No. 15: New Pogonophora from Indonesia. By Eve C. Southward. Pp. 441-452. (Sydney: The Australian Museum, 1975.) 50c. [27]
- Environmental Isotope Data No. 5: World Survey of Isotope Concentration in Precipitation (1970-1971). (Report from a network organized by the International Atomic Energy Agency, in co-operation with the World Meteorological Organization and co-operating national laboratories. Technical Reports Series No. 165.) Pp. xvii + 309. (Vienna: IAEA; London: HMSO, 1975.) 230 schillings; £5.80; \$14; DM33. [37]
- Publications du Centre National pour l'Exploitation des Océans (CNEXO). Serie: Rapports Economiques et Juridiques. No. 3: La France et le Droit de la Mer. Presentation par R. J. Dupuy, et al. Pp. 232. (Paris: Cedex: CNEXO, Boite Postale 107, 1975.) [37]
- Australian Academy of Science. Report No. 18: Report of a Working Group on Diet and Coronary Heart Disease. Pp. 72. (Canberra: Australian Academy of Science, 1975.) [77]
- Environmental Policy and Law, Vol. 1, No. 1, June 1975. Pp. 1-48. Subscriptions: Institutional etc. Sw. fr. 90 (approx. US \$36); Individual etc. Sw. fr. 50 (approx. US \$20). (Lausanne: Elsevier Sequoia; S.A., P.O. Box 851, 1975.) [77]
- CERN—European Organization for Nuclear Research. Annual Report for 1974. Pp. 222. CERN-75-5: A Study of Economic Utility Resulting from CERN Contracts. By H. Schmied. Pp. 27. (Geneva: CERN, 1975.) [77]

- Australia: CSIRO. Annual Report of the Wheat Research Unit, 1973/1974. Pp. 18. (North Ryde, NSW: CSIRO, 1975.) [77]
- University of California, San Diego. Annual Report of the Scripps Institution of Oceanography for 1974. Pp. 68. (San Diego, Calif.: UCSD—SIO, 1975.) [77]
- Australia: Material Research Laboratories. Organic Sulphur Compounds of Natural Derivation—a Bibliography. By M. W. Jarvis and K. J. Ward. 7 of 7 microfilms. (Maribyrnong: Materials Research Laboratories, 1975.) [87]
- Institut für Atomenergi, Kjeller. Kjeller Report No. 152: The Rasmussen Report Re-reviewed. (A critique of "Preliminary Review of the AEC Reactor Safety Study" prepared by the Union of Concerned Scientists, Sierra Club Joint Review Committee, November 1974.) By Jan M. Døderlein. Pp. 37. (Kjeller, Norway: Institut für Atomenergi, 1975.) [87]
- Annals of the South African Museum. Vol. 67, Part 2: A New Diacyonodent Reptile from the *Tapinocephalus* Zone (Karoo System, Beaufort Series) of South Africa, with Evidence of the Jaw Adductor Musculature. By Michael A. Cluver. Pp. 7-23. R.2.20. Vol. 67, Part 3: Taxonomic Status of the Pygocephalomorph Crustacea from the Dwyka 'White Band' (Permo-Carboniferous) of South Africa. By Brian Kensley. Pp. 25-33. R.1.70. (Cape Town: South African Museum, 1975.) [97]
- Suomen Geodeettisen Laitoksen Tiedonantoja. (Reports of the Finnish Geodetic Institute.) 75:3: On the Determination of Elliptic Orbits from the Time Micrometer Observations. By Ossi Ojanen. Pp. 12. 75:4: Measurements of Wave Motion in the Ice Surface. By Aimo Kiviniemi. Pp. 13. 75:5: Land Uplift in Finland on the Basis of Sea Level Recordings. By Erkki Kaariainen. Pp. 14. (Helsinki: Geodeettinen Laitos, 1975.) [97]
- To Conquer Hunger: Opportunity and Political Will. By W. David Hopper. (Text of a lecture delivered in the John A. Hannah International Development Lecture Series, Michigan State University, East Lansing, Michigan, May 16th, 1975.) Pp. 24. (Ottawa: International Development Research Centre, Box 8500, 1975.) [107]
- National Research Council Canada. Report of the President, 1974/1975. Pp. 77. (Ottawa: National Research Council Canada, 1975.) [107]
- CERN—European Organization for Nuclear Research. CERN 75-7: A History of the Collaboration Between the European Organization for Nuclear Research (CERN) and the Joint Institute for Nuclear Research (JINR), and with Soviet Research Institutes in the USSR, 1955-1970. By W. O. Lock. Pp. 44. (Geneva: CERN, 1975.) [107]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-30: Offshore Geology of Eastern Canada. Vol. 2: Regional Geology. Edited by Willem J. M. van der Linden and John A. Wade. Pp. ix+258: Map holder 1. (Ottawa: Information Canada, 1975.) \$12. [107]
- Tatigkeitsbericht Deutsche Forschungsgemeinschaft 1974. Pp. 292. Programme und Projekte Deutsche Forschungsgemeinschaft 1974. Pp. 682. (Bonn-Bad Godesberg: Deutsche Forschungsgemeinschaft, 1975.) [117]
- Urban Emergency Medical Service of the City of Leningrad. By M. A. Messel. Translated from the Russian Language and Reproduced in Limited Quantities by the Geographic Health Studies Program of the John E. Fogarty International Center for Advanced Study in the Health Sciences. Pp. xiii+287. (Bethesda, Md.: US Department of Health Education and Welfare, Public Health Service, National Institutes of Health, 1975.) [147]
- Lawrence Livermore Laboratory, University of California. Laser Program Annual Report—1974. Edited by James I. Davis and Wallace Clements. Pp. v+513. (Prepared for US Energy Research Development Administration.) (Livermore, California: Lawrence Livermore Laboratory, 1975.) [147]
- Smithsonian Contributions to Zoology, No. 189: An Annotated List of the Marine Mollusks of Ascension Island, South Atlantic Ocean. By Joseph Rosewater. Pp. iv+41. \$1.25. No. 190: Pycnogonida of Western Australia. By C. Allan Child. Pp. iv+29. 95 cents. No. 191: Cyclopoid Copepods (Ichthyomolgidae) Associated with Alcyonaceans in New Caledonia. By Arthur G. Humes. Pp. iii+27. No. 196: Calanoid Copepods of the Family Euchaetidae from the Gulf of Mexico and Western Caribbean Sea. By Taisoo Park. Pp. iii+26. No. 198: Ctenuchid Moths of *Ceramidia* Butler, *Ceramidiodes* Hampson, and the Caca Species Group of *Antichloris* Hubner. By William D. Field. Pp. iii+45. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [157]
- General Electric Company of the USA. Research and Development Review for 1975. Pp. 44 (Schencetady, NY: Corporate Research and Development, General Electric Company of the USA, 1975.) [157]
- The Utility of Computers in Landscape Planning: The Selection and Application of a Computer Mapping and Assessment System for the Metropolitan Landscape Planning Model (METLAND). By Kimball H. Ferris and Julius Gy. Fabos. (Bulletin No. 617.) Pp. 116. (Amherst, Mass.: Massachusetts Agricultural Experiment Station, College of Food and Natural Resources, University of Massachusetts, 1974.) [157]
- World Health Organization. Primary Pulmonary Hypertension—Report on a WHO meeting, Geneva, 15-17 October 1973. Edited by S. Hatano and T. Strasser. Pp. 45. Sw. fr. 10. Community Water Supply and Excreta Disposal Situation on the Developing Countries—a Commentary. By C. S. Pineo and D. V. Subrahmanyam. (WHO Offset Publication No. 15.) Pp. 41. Sw. fr. 12. (Geneva: WHO; London: HMSO, 1975.) [167]